

1 Global Analysis of SAR Gene Expression Reveals Promoters of Genes Co-regulated with PR1 are Enriched for W-boxes

Bob Dietrich¹, Kay Lawton¹, Klaus Maleck¹, Allen Morgan¹, Jurg Schmid¹, Aaron Levine², Jeff Dangl²

¹Novartis Agribusiness Biotech. Research Inc, Research Triangle Park, NC 27709,

²Dept. of Biology and Curriculum in Genetics, Univ. of North Carolina, Chapel Hill, NC 27599

Activation of Systemic Acquired Resistance (SAR) by biological and chemical inducers results in enhanced broad-spectrum disease resistance. A number of gene families which show increased transcript accumulation in resistant tissue following SAR induction have been identified. One gene, PR1, has provided a useful molecular marker for the SAR response. Transgenic and mutant Arabidopsis plants that are compromised at specific steps in SAR signaling (NahG, nim1) or constitutively activated for SAR (cim) have also been identified. To uncover additional changes in SAR gene expression, a pilot project has been carried out using a cDNA microarray system in collaboration with Synteni. This technology allows the detection of differences in individual transcript levels between two samples for thousands of genes in a single hybridization experiment. Analysis of changes in gene expression on a genomic scale provides a useful tool for gene discovery and mutant characterization. Expression profiles following various pathogen and chemical treatments in wild type, mutant and transgenic plants were used to compare the global effects of the treatments. Cluster analysis of gene expression changes was conducted to identify groups of co-regulated genes (i.e. regulons) during SAR. One regulon is composed of genes that are coordinately regulated with PR1. Analysis of the 5' regulatory region of genes in this regulon for which genomic sequence is available revealed the presence of functionally defined elicitor-response elements of the W box type. The W box is a common binding site for WRKY transcription factors, proteins that have been shown to be involved in regulation of early defense-response gene expression. Furthermore, in 1kb of 5' promoter sequence from these genes there is an average of 4.3 WRKY elements (i.e. W boxes) per promoter. In contrast, in 1kb 5' promoter sequence from randomly selected genes that do not cluster with PR1, less than two WRKY element occurs on average in each promoter. Statistically, two WRKY elements are predicted to occur randomly per kb of sequence.

2 Genetic basis of the winter-annual habit and control of flowering by vernalization

Scott Michaels and Richard Amasino

Department of Biochemistry University of Wisconsin 433 Babcock Drive Madison, WI 53706-1544

In contrast to the early-flowering (summer-annual) ecotypes of Arabidopsis favored for most studies, many ecotypes of Arabidopsis (winter-annual forms) are quite late flowering unless flowering is promoted by prolonged exposure to low temperatures (vernalization). This natural variation has been used as a tool to study the molecular-genetic basis of vernalization in Arabidopsis. Crosses between early- and late-flowering ecotypes have revealed that two dominant genes, FLOWERING LOCUS C (FLC) and FRIGIDA (FRI), are primarily responsible for the late-flowering, vernalization-responsive phenotype of the late-flowering ecotypes. Early-flowering ecotypes contain non-functional alleles of FRI, FLC, or both genes, and thus do not require vernalization

for early flowering. FLC acts as an inhibitor of flowering and requires the presence of FRI for high expression. The upregulation of FLC by FRI is suppressed by vernalization, and the downregulation of FLC by vernalization is stable for the duration of the plant's life. FLC levels are also upregulated in a vernalization-reversible manner in plants lacking FRI but containing autonomous-pathway null mutations. Thus vernalization acts independently of FRI and autonomous-pathway genes through a separate vernalization pathway that directly regulates FLC expression. Plants containing a constitutively expressed 35S:FLC construct remain late flowering after cold treatment, indicating that FLC levels must be downregulated for vernalization to be effective. Thus the epigenetic downregulation of FLC appears to be a major target of the vernalization pathway and provides a molecular marker of the vernalized state.

3 The evolution of floral developmental mechanisms

Vivian F. Irish

Yale University, New Haven, CT 06520

Studies of model systems like *Arabidopsis* are carried out in part because we believe that what we learn about the biology of this model system can be applied to other, less genetically tractable, species. To what extent, though, are the lessons that we learn from *Arabidopsis* directly applicable to other systems? My lab is interested in understanding how flowers develop, and how the developmental programs specifying floral organ identities have been modulated over evolutionary time to produce the varied floral forms that we see in the over 250,000 species that make up the angiosperms. Work in *Arabidopsis* and *Antirrhinum* led to the development of the ABC model of floral organ identity specification (Coen and Meyerowitz, 1991); the fact that homologs of the ABC genes have now been found in a number of other species has prompted many researchers to assume that the ABC model can explain floral development across all of the angiosperms. Is this a valid assumption? I will discuss work from my lab that suggests that the B group genes are expressed in different patterns in different angiosperm lineages, suggesting that the mechanisms operating to specify petal identities may be distinct in these different lineages. In addition, approaches to begin to critically test the idea that B group gene functions have diverged in different lineages will be presented.

4 A High Throughput Platform for Characterizing the Growth and Development Phenotypes of *Arabidopsis* Mutants

Douglas C. Boyes, Amy J. McCaskill, Angela Centra, Alexis Edwards, Timothy A. Sullivan, Nicolle Babocsi, Donald Todd, Jr., Erica M. Berglund, J. Cameron Mitchell, Robert Ascenzi, Adel Zayed, Neil E. Hoffman, Keith R. Davis and Jörn Görlach
Department of Plant Research, Paradigm Genetics, Inc., Research Triangle Park, NC 27709

As the *Arabidopsis* genomic sequencing project nears completion the next challenge is the large-scale determination of gene function. To meet this challenge, we have developed a high throughput phenotypic analysis platform in which data from over 100 traits representative of all stages of *Arabidopsis* growth and development are collected using an industrialized process. The platform consists of two major components. The first is the physical infrastructure required for growing the plants and collecting data. Plants are grown in custom walk-in growth rooms and data are collected at a series of

experimental workstations. Each workstation is optimized for a different data collection activity and the plants pass from one station to the next during the data collection process. Plant and sample tracking, as well as the steps in the data collection process, are controlled with a customized laboratory information management system (LIMS). The second component of the platform is a series of defined growth stages that serve as developmental landmarks. These growth stages allow a more robust comparison of different plants and also serve as triggers for data collection activities. The key growth stages utilized in our platform were adapted from the BBCH code¹ and are defined below:

Stage 1.04: 4 rosette leaves >1mm in length

Stage 1.10: 10 rosette leaves >1 mm in length

Stage 5.10: First flower buds visible

Stage 6.00: First flower open

Stage 6.50: 50 percent of the final number of flowers have been produced

Stage 6.90: Flower production complete

Stage 9.70: Senescence complete and seed ready for harvest

We propose these growth stages as a generic means by which phenotypic data can be collected and compared within the Arabidopsis research community. We will present a more complete description of our phenotypic analysis platform including data obtained from the analysis of stock center mutants.

¹Bleiholder, H. et al. in Manual for Field Trials in Plant Protection (Ciba-Geigy, Basel, Switzerland, 1992) pp. 246-51.

5 Analysis of Functionally Analogous Disease Resistance Genes in Soybean and Arabidopsis

Tom Ashfield, Adam Henk and Roger Innes

Department of Biology, Indiana University, Bloomington, IN 47405. U.S.A.

Rpg1 and RPM1 are functionally analogous disease resistance genes (R-genes) in soybean and Arabidopsis, respectively. Alleles at these two R-gene loci respond to the same *Pseudomonas syringae* (the causative agent of bacterial blight) avirulence genes, *avrB* and *avrRpm1*. In soybean, the response to *avrB* and *avrRpm1* is conferred by two different alleles, or tightly linked genes, at the Rpg1 locus. In Arabidopsis however, response to these two *avr* specificities is conferred by a single gene, RPM1. The molecular characterization of the RPM1 and Rpg1 loci should provide valuable information on the evolution of R-gene specificities.

The Arabidopsis RPM1 gene has been previously cloned and found to contain a putative nucleotide binding site and leucine-rich repeats, features shared with several other cloned R-genes. Work is now in progress to clone alleles from the soybean Rpg1 locus. We have genetically mapped the Rpg1 locus to a cluster of previously identified resistance genes in molecular linkage group-F (RFLP:USDA-ARS:RCS map), tightly linked to the markers R45 and PHP2265.

RPM1 detects numerous related sequences in the soybean genome when used as a hybridization probe under low stringency conditions. Unfortunately, it was not practical to map the large number of loci detected. Therefore a positional approach is being taken to clone the soybean Rpg1 gene. We have prescreened for informative recombinants in more than 900 recombinant inbred lines. BACs have been isolated with Rpg1-flanking

markers which delimit a genetic interval of 0.26cM. Twelve BACs have been ordered into two contigs that flank Rpg1. Interestingly, several of the BACs contain sequences displaying homology to previously isolated NBS-LRR type R-genes, including RPM1. At least one of the NBS-LRR genes isolated from our BAC contigs is a member of a small multigene family clustered at the Rpg1 locus. We are presently attempting to close the gap between the two BAC contigs. Agrobacterium-mediated transient transformation of soybean leaves is being optimized to enable the functional testing of putative Rpg1 clones. Progress will be reported.

We thank Laura Marek, Randy Shoemaker, Dariush Danesh, Nevin Young, Khalid Meksem and David Lightfoot for assistance with BAC library screening.

6 Plant Steroid Signaling

Jianming Li 1, Kyoung Hee Nam 1, Cong Yu 1, Dionne K. Vafeado 2, Joanne Chory 2
1. Department of Biology, University of Michigan, Ann Arbor, MI 48109-1048. 2. The Howard Hughes Medical Institute, Plant Biology Laboratory, Salk Institute, La Jolla, CA 92037

Brassinosteroids (BRs) are a special class of plant steroids that are essential for normal plant growth and development. Molecular and genetic approaches have been taken to dissect the BR signal transduction pathway, leading to the identification of a few BR-regulated genes and the Arabidopsis BRI1 gene encoding a putative BR receptor. However, the signal transduction pathway between BRI1 and various BR-induced cellular responses remains unknown. Screening for additional BR-insensitive mutants identified a new locus, BIN2, that may play a regulatory function in BR signaling. Homozygous bin2 mutants displayed many phenotypes that mimic those of bri1 mutants. Both mutants are insensitive only to BRs but retain full sensitivity to other plant hormones. A suppressor screen in a det2 (a BR-deficient mutant) background uncovered another BR-related gene, SUD1. Dominant sud1 mutation suppresses mutant phenotypes of several different BR-deficient mutations and results in a higher BR sensitivity in wild-type background. Molecular cloning of both genes will provide insights into the molecular mechanism(s) of plant steroid signaling.

7 Genetics of disease resistance in Arabidopsis to crop pathogens

Eric B. Holub

Horticulture Research Int'l

Arabidopsis is generally resistant as a species to most foliar crop pathogens. This species level resistance to non-native pathogens could potentially provide durable resistance for crop improvement if examples can be found which are amenable to genetic and molecular analysis. Genetic analyses of genotype or race specific resistance (associated in crops with "boom and bust" of resistant cultivars) is beginning to reveal likely components of species level resistance. RPS4 and RPS5 are pathogen "surveillance" or so-called R-genes (naturally variable resistance genes) which were isolated from Arabidopsis and provide components of resistance to legume pathogens (*Pseudomonas syringae* pv. *pisii* and *P.s.phaseolicola*, respectively). Another example, EDS1, is a gene that is required for transducing defense responses triggered by a major subclass of R-genes (called TIR-NBS-LRR). Mutation in EDS1 renders a plant fully susceptible to

isolates of *Albugo candida* (white rust) from *Brassica oleracea*., whereas wild *Arabidopsis* appears to be universally resistant as a species to this parasite. Such examples are not sufficient for explaining species level resistance because redundancy is likely at levels of both pathogen detection (R-genes) and at downstream defense responses. For instance, *eds1* retains residual resistance to *Peronospora parasitica* (downy mildew) from brassicas. Additional defense pathways will be discussed including NDR1-mediated resistance thought to be triggered by a different subclass of R-gene (LZ-NBS-LRR), and an alternative salicylate-independent source of downy mildew resistance.

8 The *Arabidopsis* SPIKE gene may regulate cytoskeletal organization during epidermal development

Jin-Long Qiu¹, M. David Marks², Dan Szymanski¹

¹, Department of Agronomy, Purdue University, West Lafayette, IN47907 ², Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108

The cytoskeleton consists of a complex three-dimensional network of filamentous structures in the cell. In the growing interphase cell, precise microtubule and actin microfilament organization are required for normal growth. Beyond a description of cytoskeletal organization during growth, very little mechanistic information is available on its regulation. We are taking a genetic approach to identify essential proteins that affect cytoskeletal organization. Previous results indicate microtubule and microfilament-dependent activities function sequentially during *Arabidopsis* trichome morphogenesis: microtubules are required to establish cell polarity, while both microtubules and microfilaments maintain and coordinate cell expansion during trichome development. Interestingly, trichomes on the leaves of the "distorted" class of mutants are indistinguishable from those treated with drugs that disrupt the actin cytoskeleton. The most notable phenotype of "distorted" mutants is the trichome defect. Mutations in distorted group mutants might affect trichome-specific genes, or may be weak alleles of essential genes. The seedling lethal spike mutant was identified in a family screen for plants with a distorted-like trichome phenotype and severe defects in epidermal tissue organization. The spike mutation is tightly linked to a single T-DNA insertion. Southern blots indicate that the insertion is simple, and does not include a chromosomal deletion adjacent to the T-DNA. The insertion lies in an intron near the 5' end of a long open reading frame. cDNA sequence indicates the SPIKE gene encodes a highly conserved protein with similarity to a family of cytoskeletal regulatory proteins found in humans, flies, and worms. The SPIKE-like proteins are believed to assemble at integrin-containing complexes to locally regulate microfilament organization. SPIKE mRNA is detected in wild-type plants, but not in homozygous mutants. Analysis of the SPIKE gene may provide insight into cytoskeletal reorganization, cellular morphogenesis, and tissue organization in plants.

9 Integration of floral inductive signals

Miguel A. Blazquez*, Ji Hoon Ahn and Detlef Weigel

Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037, USA. *Present address: IBMCP (CSIC-UPV), Valencia, Spain

Flowering of *Arabidopsis* is controlled by environmental (light, temperature, nutrient availability) and endogenous signals (related to the age of the plant). All these factors

ultimately regulate the expression and activity of floral meristem identity genes, such as LEAFY (LFY), which are responsible for the initiation of flower development. Recent molecular genetic studies have defined the hierarchy of signaling elements in different floral inductive pathways, but the question of how the information is integrated remains unsolved. Our results show that there are two levels of interaction between pathways. Analysis of the LFY promoter under long and short days shows that gibberellins activate LFY expression through cis elements that are different from those that are sufficient for the daylength response, indicating that the LFY promoter integrates environmental and endogenous signals controlling flowering time. On the other hand, studies on the effect of growth temperature on flowering time show that while this control is mainly exerted through the "autonomous" pathway, it affects photoreceptor function. These results indicate cross talk between flowering pathways at multiple levels.

Supported by fellowships from the Spanish Ministry of Education and the HFSP (M.A.B.), by fellowships from KOSEF and the Hoffman Foundation (J.H.A.), and by grants from the NSF and HFSP (D.W.).

10 Isolation and characterization of a HUA2 interacting factor HIF1

Yulan Cheng and Xuemei Chen

Waksman Institute, Rutgers University

The floral organ identity gene AGAMOUS (AG) plays an important role in establishing the morphology of the inner two whorls of Arabidopsis flowers. It likely functions in combination with other genes. HUA1 and HUA2 were isolated from an enhancer screen using the weak ag-4 allele and have been shown to be new members of the AG pathway. HUA2 was cloned using a map-based approach and was found to encode a novel protein with multiple signature motifs and proline-rich regions. To look for additional components in the pathway, we performed a yeast two-hybrid screen and identified several HUA2-interacting factors (HIFs). One of these factors, HIF1, is a novel protein that contains two WW domains. HIF1 can bind to the C-terminal proline-rich region of HUA2, which is consistent with previous findings that the WW domain recognizes and binds to proline-rich motifs. Northern blot analysis shows that HIF1 is expressed similarly in different genetic backgrounds (wild type, ag-3 and hua1-1 hua2-1) and different tissues (leaves and flowers). In order to find out if HIF1 plays a role in flower development, we screened T-DNA targeted lines and obtained a HIF1 knockout mutant, hif1-1. The genetic and molecular characterization of hif1-1 will be presented.

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

Ung Lee, Hye Ryun Woo, Pyung Ok Lim, Joon Hyun Park, and Hong Gil Nam

National Research Laboratory for Plant Senescence, Division of Molecular Life Sciences, Pohang University of Science and Technology, 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 are isolating genes controlling leaf senescence in *Arabidopsis* by reverse and forward genetic approaches. For reverse genetic approach, we isolated 1,000 senescence-associated cDNA clones by PCR-based suppression subtractive hybridization method. Sequence analyses of the 132 non-redundant clones indicate that 76 clones have significant homology with known plant genes in the public database and, of these, 26 clones have previously been shown to be upregulated in senescing leaves. The remaining 56 clones have as yet no functional annotation. When we have screened the unknown clones by RNA gel blot analysis, more than 60% of the clones tested appear to represent genes that show increased expression in senescing leaves. We are investigating the functions of these novel clones by systemically generating antisense transgenic lines. We will report the progress.

For forward genetic approach, we have isolated several delayed senescence mutants either from T-DNA insertional lines or chemical-mutagenized lines. We are conducting cloning of some of the delayed senescence mutants. We have identified putative clones for the *ore4* and *ore9* mutants. We will report the progress.

12 Amino acid sensing and signaling in *Arabidopsis*

Coruzzi, G (1), Brenner E (1), Chiu J (1), Hsieh MH (1,3), Kouranov A (1), Lam HM (2), Lancien M (1), Oliveira I (1,4) and Shin M (1)

(1) New York University, Dept of Biology, New York NY 10003 (2) Chinese University of Hong Kong, Shatin NT, HK, (3) Current address Mass General Hospital, Boston MA, (4) Current Address: EI DuPont, Wilmington DE

Our studies concern the structural and regulatory genes that control the assimilation of inorganic nitrogen into amino acids in *Arabidopsis*. We have shown that light induces the assimilation of N into glutamine and represses asparagine synthesis. The light regulation of the cognate biosynthesis genes, glutamine synthetase (GLN2) and asparagine synthetase (ASN1) is at least partially mediated by changes in levels of C and N metabolites. Light activates transcription of GLN2 and represses transcription of ASN1, while sucrose can induce expression of GLN2 and repress expression of ASN1, in the absence of light. We have also shown that amino acids can antagonize these sucrose-induced changes in GLN2 and ASN1 expression. These results suggest that plants can sense internal levels of carbon and/or amino acids, which in turn activates or represses expression of these specific N-assimilatory genes. Testing this metabolic sensing model, defining it mechanistically, and identifying components thereof, is the focus of our research. We have begun to characterize components of carbon and amino acid sensing/signaling mechanisms in *Arabidopsis* using reverse and forward genetics. Our reverse genetic studies are driven by the hypothesis that amino acid sensing is primitive and conserved in evolution. In support of this, the repression of GLN2 expression by amino acids in plants is mechanistically reminiscent of the Ntr system in *E. coli*. We identified an *Arabidopsis* homologue of an Ntr component, PII, and showed using transgenic plants that PII may play a role in sensing C:N levels. We also identified putative glutamate sensors, plant homologues of glutamate receptors (iGluRs) which function as ligand-gated ion channels in animal brains. Our studies on plant GluRs suggest they may play a role in light signal transduction. In addition to the above reverse genetic studies, we have developed positive genetic selections to isolate *Arabidopsis* mutants defective in sensing carbon or amino acids. We have isolated several sugar

insensitive (sin) *Arabidopsis* mutants and are conducting a screen for amino acid insensitive mutants. The molecular characterization of these carbon and amino acid sensing mutants should allow us to identify components involved in metabolic regulation of N-assimilation in plants.

13 Phototropism: Light-perception, signal transduction, hormone responses, and changes in gene expression

Emmanuel Liscum, Thomas J. Campbell, Renee M. Harper, Andrei V. Motchoulski and Emily L. Stowe-Evans

Division of Biological Sciences, 105 Tucker Hall, University of Missouri, Columbia, MO 65211

Mutational analyses in *Arabidopsis thaliana* have provided significant new insights into the molecular mechanisms mediating phototropic responses of higher plants. Null mutations in the NPH1 and NPH3 loci result in seedlings that are aphototropic to low fluence rate unidirectional UV-A/blue/green light cues, suggesting that NPH1 and NPH3 function genetically close to each other. NPH1 has been found to encode a light-activated ser/thr protein kinase that utilizes two non-covalently-bound FMN molecules as chromophores. Together with the characteristics of *nph1* mutants, these findings indicate that *nph1* (now designated phototropin) represents a new type of photoreceptor that mediates perception of directional light signals. NPH3 has been found to encode a novel protein that exhibits a light-dependent interaction with phototropin, consistent with NPH3's apparent close genetic interaction with *nph1*. We hypothesize that NPH3 acts as a scaffold/adaptor protein to bring together components of an early-acting multimolecular signaling complex that regulates the lateral redistribution of the plant hormone auxin. While NPH3 appears to be important for phototropism in low fluence rate light, another member of the NPH3 protein family, RPT2 (identified in the laboratory of K. Okada), appears to function in high fluence rate conditions. Hence, components of the proposed multimolecular signalosome appear to vary depending upon the light environment. Lesions in a fourth locus, NPH4, result in defects in multiple differential growth responses. Thus, NPH4 appears to function downstream of phototropin and NPH3, likely after the establishment of a differential auxin gradient. NPH4 encodes the auxin-responsive transcriptional activator ARF7, suggesting that localized induction of gene expression is at least one of the results of the proposed phototropin-NPH3-dependent establishment of a differential auxin gradient. We are currently using a variety of approaches to elucidate additional components of the phototropic signal-response system.

This work was supported by grants from NSF (MCB-9723124), USDA-NRI (96-35304-3709, 99-35304-8060), and UM Research Board (RB96-055)

14 Plasmodesmata exhibit two modes of intercellular movement of macromolecules, targeted and non-targeted, according to parameters imposed by subcellular localization, developmental state, and environment

Katrina Crawford, and Patricia Zambryski

Department of Plant and Microbial Biology, Koshland Hall, University of California, Berkeley, CA 94720

A quantitative assay for cell-to-cell communication in plants reveals that a striking number of cells transport large proteins through plasmodesmata. The leaf is exposed as a heterogeneous mix of cells with varying degrees of symplastic continuity, negating the view that only targeted proteins traffic plasmodesmata. Plasmodesmata are intrinsically dynamic with conformations fluctuating between closed, open and dilated. Superimposed upon this innate variation is the transport of targeted proteins (exemplified by viral movement proteins) that localize and traffic via plasmodesmata and facilitate the movement in trans of macromolecules. Non-targeted protein movement occurs by diffusion according to the aperture of plasmodesmata. Such diffusion fluctuates temporally and is restricted by protein size. Growth conditions and stage of leaf development dramatically affect the population size of cells trafficking macromolecules. A primary determinant of non-targeted transport is subcellular localization, as cytoskeletal-anchored and endoplasmic reticulum-sequestered GFP fusions do not move between cells. In contrast, nuclear-localized and cytoplasmic fusions freely diffuse. These results document two types of protein transport, targeted, and non-targeted, which may direct non-cell-autonomous development

15 Genetics of the checkpoint response to DNA damage

Sasha B. Preuss and Anne B. Britt

University of California, Davis

Treatment of seeds with very high doses of gamma radiation results in the production of "gamma plantlets". These miniature plants germinate at normal rates and exhibit normal responses to changes in their environment but cease their growth at an early stage. The arrest occurs at a stage corresponding to the presence of the plant organs at the time of irradiation, i.e., irradiated Arabidopsis seeds produce cotyledons, but not true leaves, as the mature embryo has cotyledons, but not leaves. It is possible that this phenomenon reflects the arrest of cell division in response to DNA damage. Some DNA repair defective mutants of Arabidopsis (i.e., *uvh1*) exhibit the gamma plantlet response at extremely low doses of radiation; in these mutants the arrest of cell division is clearly the result of persisting damage to DNA. Our preliminary characterization indicates that the irradiated *uvh1* cells arrest in G2. In an effort to better understand the significance and mechanism of this putative checkpoint response, our laboratory has initiated a search for second site suppressor mutants of *uvh1* that lack the gamma plantlet response. These sog (suppressor of gamma) mutants continue to undergo cell division in spite of persisting DNA damage. The existence of this mutant class indicates that the observed arrest is due to a regulatory response imposed upon the cell through a signal transduction pathway, rather than an intrinsic effect of DNA damage.

16 Root system regulation by environmental signals

Rita Martinez¹, Philip Benfey² and Jocelyn Malamy¹

¹ Molecular Genetics and Cell Biology, University of Chicago, R312 BSLC, Chicago, IL 60637 ² Biology Department, New York University, 1009 Main Bldg., 100 Washington Sq. East, NY, NY 10003

Our research focuses on the molecular regulation of root system development in *Arabidopsis thaliana*. Plant root systems are developmentally plastic, and respond to environmental conditions by adding lateral roots to the system at appropriate locations.

To produce a lateral root, a small population of mature cells in the parent root must be stimulated to proliferate and re-differentiate to form the new organ. We are interested in two aspects of this process: 1) How cell proliferation and re-differentiation is triggered in a limited manner to produce the lateral root; and 2) How the signaling pathways function that link environmental cues to organogenesis. To address these questions, we studied the effect of growth conditions on root system development. We found that specific nutritional conditions can inhibit the initiation phase of lateral root formation. To study this regulatory mechanism, we isolated mutants that overcome this inhibition. The genes affected in these mutants must be involved in the signaling pathway(s) that link lateral root initiation to environmental cues, and should provide tools to dissect the initiation process. We also found that when seedlings are subjected to osmotic stress, lateral root primordia form but never develop into lateral roots. To understand this second regulatory checkpoint we isolated mutants that fail to regulate lateral root development in response to osmotica. Molecular and physiological characterization of wild-type plants and both sets of mutants will be presented.

17 Expression Analysis of the ZIP Family of Metal Transporters

N. Grotz and M.L. Guerinot

Dartmouth College, Hanover, NH 03755

A novel family of metal transporters, the ZIP family, first identified in Arabidopsis, includes representatives from a diverse array of eukaryotes. Members of this family are able to transport a variety of metal cations including iron, zinc, manganese and cadmium. Engineering plants to bioaccumulate specific metals requires that we first understand where each of the ZIP transporters functions and how each responds to individual nutrient deficiencies. For instance, many ZIP transporters are predicted to localize to the plasma membrane while others have putative targeting signals for specific organelles. One might expect that a transporter responsible for taking metal up from the soil would be expressed only in epidermal cells. In contrast, a protein involved in transporting metal within a cell might be expressed in a broader set of tissues. In order to understand how each of the ZIP transporters participates in metal uptake in Arabidopsis, we have begun a detailed analysis of ZIP gene expression using Northern, RT-PCR and in situ hybridization techniques.

In Arabidopsis, the transcripts of some ZIP genes accumulate in response to specific metal deficiencies. IRT1 is induced in iron-deficient roots. Three zinc transporters, ZIP1, ZIP3 and ZIP4, all respond to zinc deficiency. ZIP1 and ZIP3 are induced in zinc-deficient roots while ZIP4 is expressed in both zinc-deficient roots and shoots. Northern analysis and RT-PCR have shown that ZIP3 and ZIP4 respond to iron deficiency as well as zinc deficiency. In contrast, IRT1 responds to iron deficiency but not to zinc deficiency.

In situ hybridization has revealed that IRT1 is expressed in the epidermal cells of iron-deficient roots, supporting a role for IRT1 in transporting iron from the rhizosphere. We have also examined the expression pattern of IRT1 in a mutant, *frd3*, that was originally identified by misexpression of ferric-chelate reductase activity. Wildtype plants have an iron deficiency-inducible ferric-chelate reductase activity, whereas, in a *frd3* mutant, this activity is constitutive. IRT1 is expressed in the roots of both iron-sufficient and iron-deficient *frd3* plants. Interestingly, the message is only detected in epidermal cells. This

suggests that *frd3* is specific to the iron response pathway and that there is not simply a global misregulation of IRT1 in *frd3*.

18 Organization and Function of the Ubiquitin/26S Proteasome Proteolytic Pathway in Arabidopsis

Richard D. Vierstra, Paul Bates, Jed Doelling, Adam Durski, Hongyong Fu, Pierre-Alain Girod, Mina Kurepa, Ning Yan, and Jan Smalle

Cellular and Molecular Biology Program and the Department of Horticulture, University of Wisconsin-Madison, Madison, WI 53706

Selective removal of intracellular proteins plays an integral role in the growth, development, and environmental adaptation of plants. One essential proteolytic pathway targets proteins for degradation by covalently tagging the proteins with one or more ubiquitins, a small highly conserved proteins. These ubiquitinated proteins are then selectively broken down by the 26S proteasome with the concomitant release of free ubiquitins. The complexity of the ubiquitin/26S proteasome pathway is best illustrated by estimates that 10% of plant proteins are targets and by our discovery of over 175 genes in *Arabidopsis thaliana* that encode pathway components. Correct substrate recognition is accomplished by: (1) the coordinated action of ubiquitin-conjugating enzymes (or E2s) and ubiquitin-protein ligases (or E3s) that ligate ubiquitin to specific proteins; (2) enzymes that remove ubiquitins from various targets (UBPs); and by (3) factors within the 26S proteasome that detect ubiquitinated proteins before degradation. Large structural diversity within the E2, E3, and UBP protein families is apparent that likely imparts much of this target specificity. To understand this diversity, we are analyzing the functions of various pathway components by reverse genetics, using *Physcomitrella patens* and *Arabidopsis* as model systems. Knockouts in E2s, E3s, UBPs and various 26S proteasome subunits have been generated. Phenotypic analyses show that the ubiquitin/26S proteasome pathway is essential to plants and has important roles in embryogenesis, meristem development, plant growth, hormone signaling, and various stress responses. Recently, four other polypeptides have been discovered that also serve as covalently modifiers of other plant proteins. One appears to have an essential role in nitrogen recycling. The complexities of these pathways suggest that protein modification by other polypeptides is a common method of post-translational regulation in plants. Supported by grants from USDA-NRICGP and DOE to RDV and a NIH postdoctoral fellowship to JD.

19 Comparisons of probe labeling techniques for Arabidopsis microarrays

Accerbi M., Ruuska S., Green P., Ohlrogge J., Wisman P.

Michigan State University, East Lansing, MI, 48824, U.S.A.

Microarrays are becoming increasingly popular in plant genomic research and the Arabidopsis Functional Genomics Consortium (AFGC) is providing the research community with the opportunity to take advantage of this technology. Currently, many probe labeling procedures for microarray experiments require 100 µg total or 1 µg polyA⁺ RNA. To identify possible differences in the labeling techniques, total and polyA⁺ RNA from the same source were compared. The pilot experiment indicated that approximately 10% of the clones were differentially expressed. Microarray experiments are sometimes limited by the ability to isolate large amounts of RNA from a single tissue.

To this end, commercially available alternative labeling methods, which use smaller amounts of RNA were tested. Encouragingly, a new technique requiring less than 20 µg of total RNA labeled with "dendromer" fluorescent complexes produced good initial results with our cDNA arrays. The correlation coefficients between the fluorescence intensity values of the poly A+ probes and "dendromer" probes were approximately 0.8. This project is funded by the NSF.

20 Establishment of RNA directed promoter silencing systems in *Arabidopsis thaliana*

Werner Aufsatz, Tatsuo Kanno, Florian M. Mette, Johannes van der Winden, Marjori Matzke and Antonius J.M. Matzke

Austrian Academy of Sciences, Institute of Molecular Biology, Department of Plant Molecular Genetics, Billrothstraße 11, A-5020 Salzburg, Austria

Homology-dependent gene silencing can occur both transcriptionally and post-transcriptionally, with DNA de novo methylation playing a role in both processes. Because of the sequence specificity of these processes, nucleic acid interactions such as DNA-DNA or RNA-DNA pairing are likely to be involved in triggering DNA methylation. A recent study showed that the production of aberrant promoter transcripts in tobacco contributes to methylation and transcriptional silencing of unlinked promoters in trans (1). The formation of a double-stranded hairpin promoter RNA has been implicated in triggering the silencing of the target promoter. In this work, one constitutive and two seed-specific promoters were chosen as targets for silencing in *Arabidopsis*. Appropriate homozygous target lines were supertransformed with two classes of silencing constructs: One class of constructs is able to produce overlapping antisense and sense RNAs of the target promoter from the same DNA molecule while the second class of constructs is designed to transcribe an inverted repeat of the target promoter with a heterologous linker between the repeats. Both construct classes are therefore potentially able to form double-stranded RNA homologous to the target promoter but only the second class is capable of generating double stranded hairpin promoter RNA. Results on the ability of the different silencing constructs to induce methylation and silencing of the target promoters will be presented.

(1) Mette, M.F. et al. (1999), EMBO J. 18: 241-248

21 Relocation of pericentromeric heterochromatin to an interstitial knob is accompanied by an alteration of flanking euchromatin into heterochromatin

Hoda Badry Mohammed Ali, Ingo Schubert and Paul Fransz

Department of Cytogenetics, Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, 06466, Gatersleben, Germany

A heterochromatic knob hk4S has been detected in the short arm of chromosome 4 in the *Arabidopsis* accessions Columbia (Col) and Wassileskija (WS), but not in C24, Landsberg (Ler and LER) and NoO. This knob spans 700 kb and consists of many repeat elements, which are similar to typical centromeric repeats. The polymorphism suggests a chromosomal rearrangement in this area. This idea is reinforced by the absence of the pericentromeric repeats at the hk4S position in the knob-less accessions and the fact that the RFLP marker mi233, which flanks the knob at the distal end, is present in Col and

WS, but absent from ecotype Ler. In order to obtain more information about the putative rearrangement, we hybridized two contiguous BACs, T1J1 and T4B21 that have been mapped proximal from the knob region of ecotype Col. We found the BAC signals in WS at the same position and orientation as described in the sequence map of Col.

Surprisingly, in the ecotype LER the BACs T1J1 and T4B21 were at the same position, but showed a reversed orientation indicating an inversion event. We have extended the analysis with several BACs in and around the knob and discovered an inversion spanning a 1.4 Mbp region, covered by the BACs from F9H3 to T23N4. We conclude that an inversion event has brought pericentromeric heterochromatin sequences to an interstitial position, thus creating an interstitial heterochromatic knob. Using fiber-FISH analysis we located one of the breakpoints in the region of the BAC F9H3 nearby the marker mi233. Interestingly, we discovered a region that appears euchromatic in pachytene chromosomes of the knobless accessions, but heterochromatic in the knob-containing accession WS, suggesting that the rearrangement of the pericentromeric heterochromatin segment has altered the euchromatic constitution of the flanking segment. The results will be discussed in relation to heterochromatinization and possible position effect.

22 Characterization of Gene Profile During Development Using the GeneChip

Paul Budworth, Devon Brown, Hur-Song Chang, Tong Zhu, and Xun Wang

Novartis Ag Discovery Institute, 3115 Merryfield Row, San Diego, CA 92121

Using the recently designed Arabidopsis high density oligo probe array, a total of 8775 genes of Arabidopsis thaliana were surveyed for temporal and developmental expression profiling. The objective was to identify known and novel genes that are expressed in specific organs or developmental stages. Total RNA was isolated from nine samples at different developmental stages for the microarray analysis. These samples were analyzed in nine separate Genechips that included; 1) germinating seed, day 4; 2) root 2 wk; 3) root adult; 4) leaf 2 wk; 5) leaf adult; 6) leaf senescence; 7) stem; 8) immature siliques; and 9) flowers. The samples were hybridized to the Arabidopsis expression arrays and analyzed. All genes were expressed in at least one of the samples. Of interest are genes that will be constitutively expressed in all 9 tissues or only in one tissue. Such genes, which are putative targets for constitutive and tissue specific promoters, will be discussed. Cluster analysis to determine the expression patterns in a temporal and spatial manner will be discussed

23 Physical mapping and analysis of 5S rDNA arrays of a pericentromeric region of chromosome 5 of Arabidopsis thaliana

C.Cloix, S. Tutois, O. Mathieu, C. Cuvillier, M.C. Espagnol, G. Picard and S. Tourmente
UNIVERSITE BLAISE PASCAL, AUBIERE 63177 CEDEX, FRANCE

A physical map of a pericentromeric region of chromosome 5 containing a 180bp satellite block and a 5S rDNA locus and spanning around 2Mb was established using CIC YACs clones (Tutois et al, Chromosome Research, 1999, 7, 143-156). Three 5S rDNA arrays were resolved in this YAC contig by PFGE analysis and we have mapped different types of sequences, like tandem repeats and transposable elements, between these blocks. 5S rDNA units from each of these three arrays of chromosome 5 and from chromosomes 3 and 4 were isolated by PCR. Thirty eight new DNA sequences were obtained. Two types of 5S rDNA repeated units exist: the major variant with 0.5kb repeats and one with short

repeats (251bp) detected on YAC 11A3 from chromosome 3. Although the 38 sequences displayed noticeable heterogeneity, we were able to group them according to their 5S array origin. The presence of 5S array-specific variants was confirmed with the restriction polymorphism study of all the YACs carrying 5S units (Cloix et al, 2000, Genome Research, in press). We have started the analysis of the transcription of the 5S rDNA. Actually, a major transcript has been identified together with minor 5S transcripts in different tissues and developmental stages. We have determined two 5S block(s) probably producing the major and the minor 5S transcripts, by comparing sequences of 5S rDNA units with the sequence of the reverse transcription products.

24 Monitoring and Manipulating Meiotic Recombination in the Arabidopsis Genome

Gregory P. Copenhaver and Daphne Preuss

The University of Chicago

To clarify the mechanisms that control meiotic recombination, we are exploring the regulation of the number and distribution of recombination events in the Arabidopsis genome. Using the Arabidopsis quartet mutation, which causes the four products of pollen meiosis to remain attached, we demonstrated that inhibitors of DNA methylation or histone de-acetylation can have profound effects on the levels of recombination in heterochromatic regions of plant chromosomes. This initial study relied on the analysis of the segregation of DNA polymorphisms spaced at 10 cM intervals across the Arabidopsis genome. To facilitate future studies of recombination mechanisms, we have developed a rapid and reliable visual screen for recombination events. In this screen the post-meiotic expression of two different transgenic color markers (GFP and BFP) is used to assay the segregation of a chromosome segment in pollen tetrads. In plants carrying these markers in a "trans" configuration, the parental tetrads consist of two pollen grains expressing GFP and two expressing BFP. Recombination events generate tetratype or non-parental patterns. This screen makes it feasible to i) identify mutations that stimulate or repress crossing over, ii) measure gene conversion frequencies, iii) examine recombination regulation in heterochromatic regions of the genome, such as the centromeres and rDNA, iv) characterize differences in recombination throughout the plant's life-span, and v) monitor the effects of environmental stimuli, including assault by chemicals or pathogens. Because this screen is carried out on pollen, it can be used for extremely high throughput analysis of rare events.

25 Investigating the regulation of plant response to oxidative stress with Arabidopsis stress microarrays

N.A. Eckardt¹, B.K. Higgins¹, A. Schloss¹, and N.V. Fedoroff^{1,2}

¹Life Sciences Consortium and ²Biology Department, Penn State University, University Park, PA, USA. 16802

We are using cDNA microarray gene expression analysis to identify key early stress response signaling and regulatory genes and their downstream targets. We are focusing on the air pollutant ozone (O₃), which imparts an oxidative stress to plant cells. Exposure of plants to high levels of O₃ leads to rapid formation of necrotic lesions and cell death, mimicking the hypersensitive response to pathogen infection. cDNA microarrays containing 768 elements were prepared from Arabidopsis ESTs obtained through subtractive hybridization experiments employing a number of different abiotic and biotic

stresses (including O₃ exposure, salicylic acid treatment, and infection with bacterial and fungal pathogens), plus selected ESTs from the ABRC collection corresponding to known or putative stress response and primary metabolism genes. Cy3- and Cy5-labeled cDNA probes for microarray hybridization were prepared from RNA isolated from plants harvested at different times following exposure to 0.350 µl/L ozone or clean air (controls). Hybridization results show that many of the genes represented on the stress microarray were up- or down-regulated by a factor of 2.5 or more by acute O₃ exposure. Several signaling and transcription regulation genes have been identified that are induced within 30 minutes of O₃ exposure. These genes will be targeted for antisense and/or t-DNA knockout identification, and their control over downstream stress response genes tested by further microarray expression analyses of the different responses of mutant and wildtype plants under environmental stress conditions.

26 The WRKY Superfamily of Plant Transcription Factors

Thomas Eulgem, Paul J. Rushton, Silke Robatzek and Imre E. Somssich

Max-Planck-Institut f. Zuechtungsforschung, Carl-von-Linne Weg 10, D-50829 Koeln, Germany

WRKY proteins are a superfamily of plant specific transcription factors with about 100 representatives in Arabidopsis. At present there are 68 distinct WRKY sequences available in databases. The WRKY genes all appear to be single copy and are distributed over the five chromosomes. The common feature of WRKY proteins is the occurrence of one or two copies of the highly conserved WRKY domain, consisting of about 60 amino acids. This DNA-binding domain contains a C-n4-7-C-n22-23-H-n1-H/C zinc-finger structure and the absolutely conserved name-giving peptide stretch WRKYGQK of unknown function. With the exception of the WRKY domain the overall structures of WRKY proteins are highly divergent. WRKY factors are categorized into three major groups with distinct subgroups. The WRKY domain binds sequence specifically to W boxes (T)TGAC(C/T) which are functionally characterized elicitor responsive elements. Consistent with their role as transcription factors, members of the WRKY family show nuclear targeting, transactivation capability, participation in protein complexes and are subject to phosphorylation. WRKY transcription factors appear to be involved in the regulation of various physiological programs that are unique to plants. Current information suggests that WRKY factors play key roles in regulating stress-related responses including several plant defense programs and senescence. WRKY gene expression has been shown to be upregulated in response to wounding, infection with viruses, bacteria or oomycetes, by fungal and bacterial elicitors, by signaling substances such as salicylic acid as well as upon local mechanical stimulation. The induction of WRKY expression is often extremely rapid and transient, and seems not to require de novo synthesis of regulatory factors. Beside stress-related functions, WRKY factors act in plant growth such as in trichome development. Targets of WRKY factors are quite likely genes containing W boxes within their promoters which include some WRKY genes themselves, several defense-related genes of the PR type, the HvLox1 gene and the ?Amy2/54 gene. As with other large gene families, the use of modern genetic and profiling approaches to unravel the function of individual members and subgroups might help to "wrky" matters out.

27 Quality and Statistical Analysis of AFGC Microarray Data

Finkelstein, D.B., Sterky, F., Gollub, J., Keegstra, K., Miura, E., Simon, V., Somerville, S., Cherry, J.M.

Dept of Plant Biology, Carnegie Institute of Washington, 260 Panama Street Stanford CA 94305 and DOE Plant Research Lab Michigan State University 322 PLant Biology East Lansing MI 48824

At the Arabidopsis Functional Genomics Consortium (AFGC), the technical and biological sources of variability in our microarray data were assessed in two ways. The first class of experiments consisted of replication experiments. These experiments included biological replicates, in which the physiological treatments and RNA extractions were repeated. In addition, dye reversal experiments were performed. In these experiments, the RNA that was labeled with Cy3 in one test was labeled with Cy5 in a second test. As the RNA that was labeled with Cy5 in the first test was also labeled the biological significance of the ratios did not change. This experiment allowed the effects of technique and dye chemistry to be measured. The second set of experiments focused on the AFGC printing and processing sites: one at Michigan State University, Plant Biology Laboratory and another at the Carnegie Institute of Washington, Department of Plant Biology. A repeated measures ANOVA test was used to verify the reproducibility of our methods at each site. The results of these experiments will be discussed. The presentation will include a summary of the statistical analysis we have performed. The microarray data from these experiments, as is true for all AFGC experiments, will be available to the public - see afgc.stanford.edu for more information.

28 Glk genes and leaf development in maize and Arabidopsis

David Fitter and Jane Langdale

Department of Plant Sciences, University of Oxford

Golden 2 (G2) is a novel transcription factor required for photosynthetic differentiation in both C3 and C4 maize tissues. In mutant g2 maize plants, bundle sheath cell chloroplasts differentiate aberrantly and bundle sheath enzymes do not accumulate. Differentiation of both cell types is affected in C3 maize tissues, such as the husk leaves. The G2 gene product localises to the nucleus, activates transcription and homodimerises in heterologous systems.

Library screening and sequence similarity searches have revealed a second gene in maize, and similar pairs of genes in rice and Arabidopsis. In all cases, transcripts accumulate predominantly in green tissue. G2-like genes have not been found in animals or yeast suggesting a fundamental role in the development of photosynthetic cell types.

Reverse and forward genetics are being used to elucidate possible roles for the G2-like (Glk) genes in Arabidopsis and maize development and results to date will be presented here.

29 Chromosome territories in Arabidopsis interphase nuclei revealed by chromosome painting

Paul Fransz and Ingo Schubert

Department of Cytogenetics, Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, 06466, Gatersleben, Germany, Department of Plant Sciences

It is widely accepted that chromosomes occupy individual territories during interphase. These territories have been demonstrated in human nuclei by FISH with chromosome specific paints (Lichter et al., 1988, Hum. Genet. 80, 224). Close examination revealed smaller subdomains having their transcription sites towards the domain surface. The complex organisation of the human interphase nucleus, however, does not allow a simple positioning of heterochromatin domains relative to chromosome territories. Since heterochromatin and euchromatin are well distinguishable in interphase nuclei of *Arabidopsis thaliana* we investigated chromosomal territories in this model plant using FISH with BACs that cover the entire short arm and several regions of the long arm of chromosome 4. The results show that chromosome 4 occupies a specific territory during interphase. The chromosome 4 territory surrounds the heterochromatic chromocentre formed by its centromere. We propose that each chromosome occupies a territory around its own chromocentre. In some cases we observed fused chromocentres of chromosome 4 homologues with closely associated short arms. This finding indicates that physical interactions between homologues may be accompanied by compartmentalization of their heterochromatic chromocentres. The role of the chromocentres in somatic pairing of homologs will be discussed.

30 A model for chromosome organization in interphase nuclei of *Arabidopsis*:
euchromatin loops and heterochromatin chromocentres

Paul Fransz¹, J. Hans de Jong², Pim Zabel², Ingo Schubert¹

¹Department of Cytogenetics, Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, 06466, Gatersleben, Germany, ²Department of Plant Sciences, Wageningen University, Wageningen, The Netherlands

The interphase nucleus of eukaryotes represents a complex arrangement of diverse heterochromatic and euchromatic domains, each with specific nuclear functions. The former is highly condensed and genetically inert, whereas the latter is less condensed representing sites of (potential) transcriptional activity. Both domains can be distinguished as discrete individual chromosomal segments on metaphase or pachytene chromosomes. However, in interphase nuclei a clear identification of chromosomal segments is impeded by the complex organisation of the nuclear architecture, especially in eukaryotes with a large genome. We decided to investigate the heterochromatin domains in interphase nuclei of *Arabidopsis thaliana*. The DAPI-bright chromocenters of this model plant have been neglected for long time, but now appear to play a key role in the functional organisation of interphase chromosomes. Using FISH with different probe combinations, including (peri)centromeric repeats and several unique DNA sequences, we identified individual chromocentres and examined their organization. All centromeric regions are confined to these chromocentres, while telomeres are located around the nucleolus. The chromocentres 2 and 4 also contain the (silent) 45S ribosomal genes, which map to the distal end of their short arms, approximately 3 Mbp away from the centromeres. FISH analysis with BAC contigs from the interstitial regions revealed most of the euchromatin as loops around the chromocentre. The results provide strong evidence that heterochromatic segments of a chromosome are compartmentalized in one chromocentre, while euchromatin segments form loops around the heterochromatic domain spanning 100 kb up to several Mbp. Some euchromatic segments, however, compartmentalize with the chromocentre. We speculate that these segments contain

repressed genes. Based on these results we propose a model for the organization of interphase chromosomes in *Arabidopsis*.

31 Structural, molecular and functional differences in chromatin along chromosome arm 4S of *A. thaliana*

Paul Fransz^{1,2}, Susan Armstrong¹, J. Hans de Jong², Laurence D. Parnell³, Cees van Drunen⁴, Caroline Dean⁵, Pim Zabel², Ton Bisseling² and Gareth H. Jones¹

¹ School of Biol. Sciences, University of Birmingham, UK, ² Dept. of Plant Sciences, Wageningen University, The Netherlands, ³ Cold Spring Harbor Lab., USA, ⁴ E.C. Slater Institute, Univ. of Amsterdam, The Netherlands, ⁵ Dept. of Molec. Genet., JIC, UK

The structure and the functioning of a eukaryotic chromosome are basically determined by the molecular organization of the DNA sequences, giving rise to heterochromatic and euchromatic segments. The former is associated with transcriptional inactivity, whereas the latter contains potentially active genes. Since euchromatin can become heterochromatic under certain conditions, it is clear that the organization of the chromatin plays an important role in proper regulation of gene expression. To understand the organization of the euchromatic and heterochromatic components of a eukaryotic chromosome we have constructed an integrated cytogenetic map of the short arm and centromere region of chromosome 4 of *Arabidopsis thaliana*. The map, which spans approximately 9.5 Mbp, shows the detailed positions of various multi-copy and unique sequences relative to euchromatin and heterochromatin segments. This integration forms the basis for a structural and functional analysis of different chromosomal regions along the entire chromosome arm of a eukaryotic organism. A quantitative analysis of the map positions at subsequent meiotic stages revealed a remarkable pattern of spatial and temporal variation in chromatin condensation and emphasizes the structural and molecular differences between and among euchromatin and heterochromatin segments. For example, the centromere region consists of three heterochromatic domains with distinguishable structural, molecular and functional properties. We have further characterized a conspicuous heterochromatic knob, which accommodates a tandemly arranged repeat flanked by dispersed repeats, most of which are also present in the pericentromeric heterochromatin. Both tandem and dispersed repeats are likely involved in the formation of heterochromatin.

32 DDM1 is involved in the organisation of chromocentres

Paul Fransz¹, Monica Ruffini Castiglione² and Ingo Schubert³

¹Department of Cytogenetics, Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, 06466, Gatersleben, Germany, ²Department of Plant Sciences, Istituto di Mutagenesi e Differenziamento, Pisa, Italy

Chromocentres are the heterochromatic domains in interphase nuclei of *Arabidopsis thaliana*. They accommodate mainly centromeric and pericentromeric repeats as well as (silent) ribosomal genes. Using immunolabeling with appropriate antibodies we characterized chromocentres as domains with highly methylated DNA and hypo-acetylated histone H4. We observed similar morphological features in leaf material and in somatic cells from immature flowerbuds of different accessions. The *ddm1* mutant (in a Columbia and in a Landsberg background) differs from the wild types in having smaller

and highly diffuse chromocentres, suggesting a disturbed heterochromatin formation. This was confirmed by the diffuse fluorescence pattern of 5-methyl-cytosine. In addition, fluorescence in situ hybridization with centromeric and pericentromeric repeats showed dispersed signals suggesting a lower degree of compartmentalization of pericentromeric repeats with chromocentres as compared to the wild type. Also the close association between the hybridization patterns of centromere and pericentromere repeats is lost in the mutant. The results provide evidence for a direct correlation between histone H4-acetylation, DNA methylation, chromocentre organization and the formation of heterochromatin in interphase nuclei. We conclude that the chromocentres in the *ddm1* mutant are disorganized. Our data support the idea that the DDM1 protein is involved in chromatin remodelling. The significance of these findings in relation to epigenetic regulation of gene expression is discussed.

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

Introduced genes often show variable expression in different transgenic lines. This problem severely limits the use of transgenic plants in research and biotechnology. To study this phenomenon we are taking a molecular genetic approach in *Arabidopsis thaliana*.

To investigate the influence of transgene copy number on gene expression, we have studied transgenic lines carrying between one and six copies of a chimaeric glucuronidase (GUS) marker gene under the control of the 35S promoter. All lines carrying a single copy of the marker gene in the homozygous fashion showed uniformly high expression, hemizygous lines revealed an approximately two-fold reduced expression. All transformants harbouring three or more copies of the reporter gene displayed a much lower expression of the marker gene. For example, homozygous lines carrying three copies of the marker gene in tandem showed a decrease in marker gene activity with the age of plants, resulting in almost complete silencing of the reporter gene. Our results clearly demonstrate a tight correlation of reporter gene copy number and its expression. We are currently analysing to which extent the chromosomal environment of the different integration events contributes to gene silencing of transgenes. Therefore, the chimaeric GUS reporter gene has been cloned in a Ds element. Transgenic plants carrying the Ds with the highly expressed reporter gene were crossed with plants carrying an Ac transposase source. Many different germinal Ds excision events could be recovered from the F2 progeny and very few plants with a severely reduced reporter gene expression could be identified. Map positions for the Ds elements carrying the reporter genes with reduced reporter gene expression will be presented.

34 A functional analysis of the *Arabidopsis* genome using DNA microarray analysis and T-DNA insertional mutagenesis

P. Green¹, K. Keegstra¹, J. Ohlrogge¹, E. Wisman¹, S. Somerville², M. Cherry², M. Sussman³, R. Amasino³, S. Austin-Phillips³, S. Dellaporta⁴

¹Michigan State University, ²Carnegie Institute of Washington at Stanford University;

³University of Wisconsin, Madison; ⁴Yale University

The Arabidopsis Functional Genomics Consortium (AFGC), comprised of the investigators listed above, is a collaboration funded by the Plant Genome Research Program of the National Science Foundation. Our goal is to provide plant biologists 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. One approach is to assemble DNA microarrays of many of the genes of Arabidopsis and establish a service facility to provide researchers with access to these arrays. By hybridizing DNA microarrays with probes corresponding to mRNA from different tissues and organs or from plants subjected to different stimuli, global gene expression patterns can be investigated. We are also involved in the set up of database and software support to facilitate searching and comparisons among these global gene expression patterns. A second synergistic approach has been to establish a service facility that provides high throughput screening of T-DNA insertion lines for gene knockout mutations. The gene knockout facility opened last fall at the University of Wisconsin, and the DNA microarray facility, which has two sites: Carnegie Institute of Washington at Stanford University and Michigan State University, opened at the beginning of this year. Both are now providing services to the international academic community. Our website <http://afgc.stanford.edu> provides information about these facilities and another aspect of our project that focuses on plant-specific genes. The knockout facility can also be accessed directly at <http://www.biotech.wisc.edu/Arabidopsis/>

35 PlantsP: A Database for Functional Genomics of Plant Phosphoproteins
M. Gribskov¹, A.C. Harmon², J. Harper³, E.M. Hrabak⁴, J.J. Kieber⁵, D.D. Randall⁶, D.W. Smith¹, G.E. Schaller⁴, F.E. Tax⁷, J.C. Walker⁶, S. Zhang⁶

1-University of California, San Diego, 2-University of Florida, 3-The Scripps Research Institute, 4-University of New Hampshire, 5-University of North Carolina, 6-University of Missouri, 7-University of Arizona

The Functional Genomics of Plant Phosphoproteins project (NSF DBI-9975808) is a multi-institutional effort investigating the biology of protein kinases and phosphatases in *Arabidopsis thaliana* and other plants. The experimental component of the project will screen for and identify T-DNA insertional knockouts in approximately 500 protein kinases and phosphatase genes over the next three years. The identity and phenotype of these knockouts will be stored in the PlantsP database. In addition, PlantsP will comprise detailed annotation of kinase and phosphatase families, sequence patterns and motifs, protein structural features, gene structure, and a variety of additional information. Currently, PlantsP comprises approximately 1800 sequences. We are in the early stages of systematically annotating sequence features, and classifying the sequences according to their families. Our initial focus has been on the calcium dependent protein kinases (CDPK) for which we have completed extensive phylogenetic analysis. Analysis of additional families is in progress.

36 Use of DNA microarray technology to examine mRNA turnover
Gutiérrez, R.A., Wisman, E. and Green, P.J.

MSU-DOE Plant Research Laboratory. East Lansing, MI. 48824. USA

The steady state level of a transcript at any time in a cell is determined by the combination of its rate of synthesis and degradation. Hence both transcription of a gene

and post-transcriptional regulation of the stability of its mRNA, play critical roles in the regulation of gene expression.

Determining the turnover rate of a transcript and how that rate changes in response to internal or external signals is key in evaluating the contribution of post-transcriptional control mechanisms to the expression of a particular gene. Decay rates can be measured by following the disappearance of a transcript by northern blot after inhibition of transcription. Typically, a general transcription inhibitor is utilized (e.g. cordycepin) and time courses are generated by extracting tissue samples at regular intervals after the tissue has been exposed to the inhibitor. We have combined this simple experimental strategy with the power of DNA microarray technology, for the purpose of studying genome-wide patterns of mRNA decay in Arabidopsis. Although DNA microarray technology has been used in a variety of organisms to compare mRNA levels, its application to global post-transcriptional studies has been rare or absent, particularly among multicellular eukaryotes.

Using the 11K array of the Arabidopsis Functional Genomics Consortium which is printed on glass slides, we have begun to examine mRNA decay rates of Arabidopsis transcripts. Northern blot analysis of selected transcripts has been used to confirm the data. Our initial microarray studies indicate that this approach will be most effective for providing rough estimates of the rates of decay for mRNAs with half-lives of 1.5 h or less. Transcripts with the shortest half-lives are of particular interest because they provide plants with a means to respond rapidly to exogenous or endogenous stimuli. Beyond information about decay rates of various individual transcripts, this approach has the potential to reveal patterns of mRNA stability among groups of genes that can reveal new post-transcriptional regulatory networks. Supported by the USDA, NSF and DOE.

37 Genome Wide Analysis of Clock-Controlled Transcription Using High-Density GeneChip Microarrays

Stacey L. Harmer¹, Hur-Song Chang², Bin Han², Carol R. Andersson³, John B. Hogenesch⁴, Tong Zhu², Xun Wang², and Steve A. Kay¹

¹Scripps Research Institute, 10550 N. Torrey Pines Rd La Jolla, CA 92037 ²Novartis

Agricultural Discovery Institute, 3115 Merryfield Row San Diego, CA 92121

³c.andersson@cmar.csiro.au ⁴john.hogenesch@gnf.org

Like many other organisms, plants possess internal biological clocks that help them coordinate internal events with the external environment. The circadian clock allows plants to anticipate the environmental changes that occur every 24 hours, such as sunrise and sunset. The widespread presence of circadian rhythms across taxa suggests that they provide organisms an adaptive advantage. A number of circadian controlled processes have been identified in plants, including rhythmic leaf movement, stomatal opening, and hypocotyl elongation. These clock-controlled physiological changes are likely to be effected through changes in gene transcription; in fact, a considerable number of mRNAs have been reported to undergo circadianly regulated changes in abundance. However, there has been no reported systematic study of the number and types of genes that are regulated by the circadian clock in any organism. Recently, the advent of high-density DNA microarrays has made the monitoring of global changes in gene regulation possible. Microarrays have been successfully used in a number of biological systems, with applications ranging from the identification of cell cycle controlled genes in yeast to the

categorization of human tumor clinical subtypes. We have made use of high-density GeneChip microarrays composed of synthetic oligonucleotides representing a large portion of the genes encoded by the Arabidopsis genome to monitor the circadian control of gene expression. We have also examined the circadian regulation of gene expression in Arabidopsis plants overexpressing genes implicated in the control of the circadian clock. Results will be presented on the different pathways that exhibit clock regulation, along with new roles for the clock in coordinating plant metabolism and development.

38 Arabidopsis Functional Genomics at RIKEN Genomic Sciences Center
Takashi Hirayama, Minami Matsui, Motoaki Seki, Takuya Ito, Reiko Motohashi,
Masatomo Kobayashi, Miki Nakazawa, Yoshiharu Y. Yamamoto, Takanari Ichikawa and
Kazuo Shinozaki

Plant Functional Genomics Research Group, RIKEN Genomic Sciences Center
RIKEN Genomic Sciences Center (GSC) was founded two years ago aiming at comprehensive decoding of genome information from human to plant. Plant Function Genomics Research Group (Project leader; K. Shinozaki) of GSC, started last October; will aim at comprehensive analysis of plant gene function using Arabidopsis as a model at its first stage. Our group is composed of two groups, Plant Mutation Exploration Team (Team Leader; K. Shinozaki) and Plant Function Exploration Team (Team Leader; M. Matsui).

Our group will systematically analyze expression patterns of individual Arabidopsis genes using cDNA microarray technique. We are going to collect 10,000 full-length cDNAs from Arabidopsis plants grown under various conditions. We will use these cDNAs for construction of cDNA microarrayer to analyze gene expression.

This reverse genetics approach will be most powerful if it combined with mutation lines, which cover mutations in almost all genes. For this purpose, our group will prepare a complete set of mutants using T-DNA or Ac/Ds transposon system. To overcome gene function redundancy and to gain different spectrum, we are constructing a large set of activation tagging lines (100,000 lines) and Ds insertion lines (10,000 lines). We are planning to use these tagged mutants to analyze important genes involved in plant development and response to environmental conditions.

These genomic informations obtained from our reverse and forward genetics approaches will be used to construct a database on functions and expressions of Arabidopsis genes, ie, the Plant Gene Functional database. Our database will be opened to public for plant functional genomics research.

39 Mapping of 465 Ds-transposon insertion sites transposed from start lines on Arabidopsis chromosome V

Takuya Ito 1, Reiko Motohashi 2, Kazuo Shinozaki 1, 2

1 Plant Molecular Biology Lab., RIKEN, Japan and 2 Plant Mutation Exploration Team, Plant Functional Genomics Research Group, RIKEN Genomic Sciences Center, Japan
Transposons are widely used for gene-tagging approach in various organisms not only because it is easy to access to molecular analysis, but also because they can be remobilized. To generate a library of transposon insertions, we adopted an Ac/Ds local transposition system (1). We have produced Ds-transposon insertion lines (2) from 3 parental donor Ds-T-DNA lines whose loci had been mapped on chromosome V (1).

Most of the insertion lines contain a single and stable Ds element. We have recovered Ds-flanking genomic fragment by using Tail-PCR to map the insertion sites at a nucleotide level, and mapped 465 insertion sites. They were distributed to 9% on chromosome I, 19% on II, 9% on III, 20% on IV and 43% on V. This result indicates that Ds can move to unlinked position to some extent, although preferentially moves to linked position. We found 2 insertion hot spots, NOR2 and NOR4, other than 3 donor loci. About 9% were transposed to these 2 loci. Now, we are in progress to construct Insertion Site Database consisting of each 1-kb genomic DNA covering insertion site.(1) Smith et al., *Plant J.* 10,721-732.(2) Ito et al., *Plant J.* 17, 433-444.

40 Functional Genomics of Chromatin: Global Control of Plant Gene Expression
R. Jorgensen¹, V. Chandler¹, D. Mount¹, C. Pikaard², E. Richards², H. Kaeppler³, S. Kaeppler³, K. Cone⁴, S. Gelvin⁵, and J. Bender⁶
¹Univ. of AZ, ²Washington Univ., ³Univ. of WI-Madison, ⁴Univ. of MO, ⁵Purdue Univ., ⁶Johns Hopkins Univ.

Our goal is to identify and functionally analyze the entire complement of genes in Arabidopsis and maize that contribute to chromatin-based control of gene expression, utilizing a variety of reverse genetic approaches. We are capitalizing on the large number of yeast and animal chromatin genes that share similarity with sequences in the expanding Arabidopsis and maize genome databases. To disrupt chromatin gene function, we will use a dominant negative transgene strategy involving expression of a hairpin RNA to promote degradation of homologous transcripts. This strategy has the advantage that transgenes can be designed to reduce the activity of entire gene families or of specific gene family members. Furthermore, an inducible transgene promoter will allow propagation of severely deleterious or lethal mutants, and manipulation of gene function at different developmental stages. We will also screen for transposon and T-DNA insertion alleles of targeted chromatin genes. Chromatin mutant strains will be functionally characterized with a battery of assays including genetic transmission, plant growth and development, histone acetylation, DNA methylation, nucleolar dominance, stability of epimutations and paramutations, and stability of silent states of transgenes and transposons. All mutants and transgenic lines produced in this project will be deposited in Arabidopsis and maize genetic stock centers, and all gene function data will be provided to the community via a public database.

41 Arabidopsis Centromeres: Minichromosome Analysis of Functional Centromere Domains
Kevin C. Keith, Gregory P. Copenhaver, Song Luo and Daphne Preuss
Dept. Molecular Genetics and Cell Biology, The University of Chicago, 1103 E. 57th St-EBC rm 304, Chicago, IL 60637

In order to understand the regions necessary for centromere function and chromosome segregation during meiosis and mitosis, we defined the genetic material encompassing all five Arabidopsis centromeres. Combined with the availability of DNA sequence information, Arabidopsis has the most complete, contiguous nucleotide sequence of centromeric DNA in a higher eukaryotic organism, making it an excellent model organism for centromere studies. All five Arabidopsis centromeres have a similar organization: large tracts of 180 bp repeat sequences flanked by repetitive DNA that is,

surprisingly, interspersed with unique DNA, including expressed genes. In an effort to define the role of repetitive and unique centric DNA in chromosome segregation, we are developing minichromosomes, defined chromosomal segments harboring centromere fragments that segregate in a cell-autonomous manner. We will describe our approaches for designing minichromosomes and development of quantitative assays that monitor the fidelity of chromosome segregation, making it possible for the first time in a multicellular organism to directly measure chromosome loss rates during a single mitotic or meiotic event.

42 Dominant mutations from 35S Enhancer-Transposon mutagenesis

Thomas Kirch, Rüdiger Simon and Wolfgang Werr

Institute of Developmental Biology, University of Cologne, Gyrhofstr. 17, 50923 Cologne, Germany

To identify novel genes controlling the function of the shoot apical meristem, we have established a simple but efficient transposon-mediated two element activation tagging system in *Arabidopsis*. We are screening for dominant gain-of-function mutants that show an alteration of plant body, such as changes in meristem size, organ identity, phyllotaxis or dorso-ventral pattern. In contrast to (recessive) loss-of-function mutants, dominant gain-of-function mutants will enable us to identify genes with redundant functions in development, or genes whose activity during later stages is concealed by embryo lethality.

Dominant gain-of-function alleles are created by insertions of a defective I/D Spm element that 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 will isolate transposition events, that are genetically unlinked and stable, but still carry the enhancer tetramer.

The principle of the system and primary analysis of four dominant mutants will be presented.

43 *Arabidopsis* T-DNA lines as tool for systematic reverse genetic screens

H.Uener Kolukisaoglu, Burkhard Schulz

University of Cologne, Institute of Botany II, Max-Delbrueck Labor, Carl von Linne Weg 10, 50829 Koeln, Germany

We employed the different populations of T-DNA lines available from the *Arabidopsis* Stock Centers to generate a fast and reliable system for reverse genetic screens using these resources. DNA of about 33000 lines was isolated and used to screen for insertion mutants in a variety of genes involved in processes of plant metabolism and development (e.g. protein folding, signal transduction, membrane transport and metabolism). With a combination of T-DNA border primers and gene specific primers PCR reactions were performed on genomic DNAs from the insertion populations. For about 40 % of the genes of interest a T-DNA insertion could be found in the genomic region screened. Half of those insertions are located within the coding region of the genes of interest. Data obtained with this approach led to interesting insights into T-DNA integration process

into the genome. They also show that the lines distributed by the Arabidopsis Stock Centers represent valuable resources for reverse genetic screens.

44 Cluster Analysis of Arabidopsis Microarray Data

Jeff R. Landgraf, Robert Schaffer, Matt Larson, Curt Wilkerson, Pam Green, and Ellen Wisman

MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824-1312

The microarray facility at Michigan State University is part of the Arabidopsis Functional Genomics Consortium (AFGC), a collaboration funded by the National Science Foundation. Together with Shauna Somerville at Carnegie Institute and Mike Cherry at Stanford University our goal is to make DNA microarray technology available to the academic community. Microarray data collected by both the Carnegie Institute and Michigan State is deposited in a public database at Stanford (Genome-www4.Stanford.edu/MicroArray/SMD) which provides access to the data as well as analysis tools. Expression data collected thus far has allowed us to begin cluster analysis of a number of diverse experiments. Cluster analysis attempts to arrange genes according to similarity in the pattern of gene expression. This allows organization of genes into functional groups often involved in a particular biological process. In addition, it can provide clues to poorly characterized or novel genes based on grouping with genes of known function. Data from these analyses are presented.

45 Centromere Conformation and Control of Centromere-linked Gene Expression in Arabidopsis

Song Luo and Daphne Preuss

Department of Molecular Genetics and Cell Biology, University of Chicago

Centromeres undergo dramatic changes in morphology through the cell cycle, alternating between an extended conformation during cell growth and a condensed form incorporating millions of base pairs during mitosis and meiosis. Recently, our laboratory used genetic analysis to define the regions that provide centromere function in Arabidopsis; these regions undergo far less meiotic recombination than the rest of the genome. We are expanding our analysis of centromeres, determining the relationship between the primary DNA sequence and secondary structure. First, we are examining the methylation state of the entire centromere by digesting genomic DNA with methylation sensitive enzymes and with DNA sequencing methods that directly detect methylated cytosine. These experiments demonstrate that methylation levels vary across the centromeres. We are now investigating whether these differences in methylation levels affect centromere function. Interestingly, several genes are located within centromeric heterochromatin, and many of these are single-copy genes with corresponding ESTs in the genome database. We are using microarrays to monitor the expression of these genes, and in particular are correlating expression levels with the conformation of centromeric heterochromatin.

46 Targeting Induced Local Lesions IN Genomes (TILLING) for plant functional genomics

Claire M. McCallum^{1,2}, Luca Comai³, Elizabeth A. Greene¹ and Steven Henikoff^{1,4}

1 Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
2Molecular and Cellular Biology Program 3Department of Botany, University of
Washington, Seattle, WA, USA 4HHMI Laboratories

We describe a simple and general reverse genetic strategy and demonstrate its application to plants. DNA from EMS-mutagenized individuals is subjected to PCR followed by denaturing high pressure liquid chromatography analysis to screen for mutations in a gene of interest. When applied to two *Arabidopsis thaliana* chromomethylase genes, missense and truncation lesions were discovered at high frequency. "TILLING" (for Targeting Induced Local Lesions IN Genomes) has several advantages over other reverse genetic approaches, including speed and automation, reliability of chemical mutagens, applicability to both essential and non-essential genes, provision of a range of alleles and suitability for high throughput. We are currently establishing a collection of ~10,000 mutagenized reference M2 *Arabidopsis* plants for large scale TILLING, which could become a community-wide resource. Since induced mutagenesis is widely used, our strategy can even be employed in species that lack well-developed genetic tools, and has the potential of providing targeted modification of crops without the introduction of foreign DNA.

47 Use of Insertional Mutagenesis for the Identification of Essential Genes in *Arabidopsis*

J. McElver¹, G.Aux¹, J.Tossberg¹, G. Budziszewski¹, R.King², I.Tzafir², J.Levin¹, D.Meinke², D.Patton¹

¹Novartis Agribusiness Biotechnology Research Inc., Research Triangle Park, NC ²Department of Botany, Oklahoma State University, Stillwater, OK

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 100,000 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. We have used TAIL PCR to recover flanking sequence from a large collection of lethal mutants. Approximately one-third of the recovered sequences 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.

48 Analysis of gene expression under drought and cold stress using *Arabidopsis* full-length cDNA microarray

Narusaka, M. ¹, Seki, M. ^{1,2}, Carninci, P. ³, Hayashizaki, Y. ³ and Shinozaki, K. ^{1,2}
¹Plant Mut. Expl. Team, Plant Fun. Gen. Res. Gr., GSC, RIKEN, ²Lab. Plant Mol. Biol., RIKEN, ³Genome Sci. Lab. RIKEN

Two full-length cDNA libraries were constructed from drought- and cold-treated *Arabidopsis thaliana* plants using biotinylated CAP trapper method, which is based on 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. We sequenced 4387 clones from the 5' end for the analysis of cDNA libraries in 1999. Homology search of DNA sequences among the clones revealed that 1882 clones had homology with other clones and the remaining 1377 clones were independent. We searched sequence similarity of independent 1043 clones against the non-redundant nucleotide and amino acid sequence database of NCBI, using BLAST algorithm. More than 90% of the 1043 clones obtained were full-length. Among the 1043 clones, 435 clones had homology to known genes registered in the database, and 109 were novel. 39 and 26 clones that were isolated only in the cold and drought libraries, respectively, had more than five redundancy. Among these clones, 27 cDNAs code for heat shock protein, and 22 cDNAs for glyceraldehyde-3-phosphate-dehydrogenase. We also prepared Arabidopsis full-length cDNA microarrays containing about 1400 independent full-length cDNAs. The cDNA microarrays were used to monitor expression patterns of genes under drought- and cold-stress. We analyzed the correlation between stress-induced genes identified using cDNA microarray and population of cDNAs in the cold- or drought-treated libraries. We will discuss novel stress-inducible genes identified using the cDNA microarray.

49 Insertional Mutagenesis of the Aux/IAA and ARF Gene Families in Arabidopsis Overvoorde1, P.J., Hughes1, B.A., Alonso2, J.M., Ecker2, J., and Theologis1, A.
1Plant Gene Expression Center, 800 Buchanan, Albany, CA 94710 ; 2Department of Biology, U. of Pennsylvania, Philadelphia, PA 19104

The proteins encoded by the auxin regulated Aux/IAA genes are short-lived, nuclear-localized, and contain four conserved domains (I, II, III, IV). Domain III is predicted to form an amphipathic ???-fold that is found in the prokaryotic transcriptional repressors Arc and MetJ. In the prokaryotic proteins, the ???-fold mediates both dimerization and DNA binding. Although no specific DNA-binding has been yet demonstrated, domain III plays a critical role in mediating homo- and hetero-dimerization among Aux/IAA family members and the ARF proteins. The ARF family of transcription factors bind to auxin-responsive elements located in the promoters of many early auxin-inducible genes, including members of the Aux/IAA gene family. Interestingly, in addition to their N-terminal DNA-binding domain most ARF proteins contain Aux/IAA-like domains III and IV at their C-termini. Since there are at least 22 Aux/IAA and 16 ARF genes in Arabidopsis, the number of combinatorial interactions among them is huge and offers a molecular basis for the pleiotropic effects of auxin. We are employing a reverse-genetic approach in an effort to elucidate the function of each of these genes. Screening 30,000 T-DNA insertional lines using a high-throughput strategy, putative insertional mutants for more than 50% of the genes have been identified. The location of each T-DNA insertion is determined by sequencing PCR products, and the insertion lines are outcrossed and analyzed by RNA-gel blot, southern analysis, and phenotypic screens. Preliminary phenotypic analysis reveals only subtle changes in the single insertion mutants of the Aux/IAA genes indicating overlapping function among the members of this family. Construction of lines with double, triple, or higher order insertions have the potential to reveal the function of these proteins. In addition, these mutant lines can be used for global expression studies using microarray technology for identifying genes whose expression are affected by the Aux/IAA and ARF proteins.

50 Small chromosomal deletions in Arabidopsis induced by transposition of Ds element and their implications on efficiency of tagging systems

Serguei Parinov, Mayalagu Sevugan, De Ye and Venkatesan Sundaresan

Institute of Molecular Agrobiolology, 1 Research Link, The National University of Singapore, Singapore, 117604

An insertional mutagenesis system utilizing Ds element in Arabidopsis has been analyzed (Sundaresan et al. 1995; Parinov et al. 1999). In this system, the Ds element in the donor site is closely linked to a negative selection marker IAAH gene, to facilitate selection for unlinked transposition events. However analysis of sequences flanking the Ds element showed that up to 15% of transposants that passed negative selection still carried the sequences of the donor site, although they can also carry additional transposed Ds elements. We have shown that this occurs due to various deletions of genomic DNA adjacent to Ds donor site, frequently resulting in loss of the IAAH gene. The length of the deletions range from less than 1 kb to ~70 kb. In most cases deletions are likely to be the result of recombination between the transposed Ds and the donor site. The implications of these findings for the effective use of transposon based tagging systems is discussed.

Sundaresan V, Springer P, Volpe T, Haward S, Jones JD, Dean C, Ma H, Martienssen R. (1995) Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. *Genes Dev* 9: 1797-810

Parinov S, Sevugan M, De Ye, Yang WC, Kumaran M, Sundaresan V. (1999) Analysis of Flanking Sequences from Dissociation Insertion Lines. A database for reverse genetics in arabidopsis. *Plant Cell* 11: 2263-2270

51 Functional Genomics For Photosynthesis-Limiting Factors In Arabidopsis

Pesaresi, P., Varotto, C., Maiwald, D., Kurth, J., Kalde, A., Abdallah, F., Saedler, H., Salamini, F. and Leister, D.

Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné Weg 10, 50829 Köln, Germany

In 1998, the 'Centre for Functional Genomics in Arabidopsis' (ZIGIA) at the MPI/Cologne was founded. ZIGIA studies gene functions by analysing insertional mutants, using forward and reverse genetics. Genes with impact on pathogen resistance, growth, fertility and photosynthesis are primary targets of ZIGIA. Following, results of the photosynthesis part are presented. A novel screening approach was set up leading to the identification of 36 mutants affected in chlorophyll fluorescence (as indicator for photosynthetic performance). Additional 43 mutants were identified by their pigmentation phenotype. 8 mutated genes have been identified and, in part, characterised. Their gene products belong to very diverse classes, comprising photosystem sub-units, transporters, enzymes involved in chromophore biosynthesis and factors involved in protein synthesis, processing and targeting. The reverse genetics approach aims at the identification of knock-outs for genes with putative impact on photosynthetic performance. First data and results covering genome-wide databases analyses of the Arabidopsis proteome will be presented.

52 High Resolution AFLP Sequence Map of Arabidopsis thaliana

Janny L. Peters, Hans Constandt, Marc Zabeau and Tom Gerats
University of Ghent, Department of Plant Genetics, VIB, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium

To facilitate the positional cloning of genes of interest, a truly high-resolution physical map with easily genotyped markers is needed. We significantly accelerated the normally time-consuming procedure for producing a high-resolution map by combining *in silico* and experimental AFLP analysis. The nearly completed sequence of *Arabidopsis thaliana*, ecotype Columbia (Col), allows us to perform *in silico* AFLP, whereas experimental AFLP analysis identifies Col AFLP fragments that are polymorphic with Landsberg (Ler) and/or other ecotypes. We can correlate experimental and *in silico* AFLP analysis by using the fragment size and selective nucleotides as discriminating parameters and subsequently position the experimentally obtained Col AFLP markers directly onto the *Arabidopsis* sequence map. To assign each experimentally obtained Col AFLP marker to a defined segment of the *Arabidopsis* genome, we analysed seven recombinant inbred (RI) Col/Ler lines for the identified polymorphisms. A set of markers, as identified by Lister and Dean (http://nasc.nott.ac.uk/new_ri_map.html), allowed these segments to be translated from genetic segments to physical-map segments. All possible 256 SacI+2/MseI+2 primer combinations were performed. This will allow the physical localisation of approximately 2000 Col AFLP markers, i.e., 1 marker per 65 kb. Additional sets of markers can be obtained by using other restriction enzymes.

53 Exploiting the genome sequence database for rapid mapping of transposon insertion lines on *Arabidopsis thaliana*

Raina S., Mahalingam R., Chen F. and Fedoroff N. V.

The Pennsylvania State University, University Park, PA-16802

We have used the maize Ds element to generate new insertions of marked transposons in the *Arabidopsis* genome. We have amplified and sequenced insert-specific flanking sequences from 323 independent Ds transposon lines using thermal asymmetric interlaced (TAIL) PCR. The insertion sites were identified for 210 transposon lines using *Arabidopsis* genomic clones (BAC, YAC, TAC and PI) and/or ESTs for which the chromosomal sequences are defined in the database. A majority of insertions (77%) were found to be in the promoter region or with the open reading frame of various genes whose functions have been predicted by computer algorithms. Insertions were identified on all five *Arabidopsis* chromosomes, with Chromosome 1 containing the largest and Chromosome 3 the smallest number of inserts. Although transposed elements were found frequently linked to their donor loci, the transposition pattern varied considerably around the donor loci. Our results demonstrate that a systematic, high resolution mapping of transposon insertions in *Arabidopsis* can be achieved efficiently by TAIL PCR when the genome is completely sequenced. The complete database for 210 insertion sites will be available at - <http://www.lsc.psu.edu/ptl/ptlmain.html>, upon publication of these data. A large collection of *Arabidopsis* lines containing mapped transposable element will be useful for forward and reverse genetics.

54 The *Arabidopsis* Information Resource (TAIR)

Seung Y. Rhee(1),Eva Huala(1),Margarita Garcia-Hernandez(1), Leonore

Reiser(1),Allan Dickerman(2),Dan Weems(2),J.J. Zhuang(2),Andrew Tolopko(2),Wen

Huang(2),David Hanley(2),Damian Gessler(2),Dawn Perry(2),Bruno Sobral(2),and Chris Somerville(1)

1. Carnegie Institution of Washington, Department of Plant Biology, 260 Panama St., Stanford, CA 94305; 2. The National Center for Genome Resources, 2935 Rodeo Park Drive East, Santa Fe, NM 87505

TAIR (www.arabidopsis.org) is a Web-based information resource for plant researchers. Our goal is to provide accurate and up-to-date genomics resources for Arabidopsis to the scientific community. We are transitioning from AtDB's web content to new database structure and user interfaces to enhance the information content, integrity, and accessibility of data through the Web. This first phase of our project includes: 1) development of a database structure in Sybase; 2) transfer of data from AtDB to TAIR; 3) development of a user-friendly web navigation and query structure; and 4) development of a map visualization tool to graphically align and display genetic and physical map data from our database. The integrated map visualization tool, TAIR MapViewer, is currently under beta testing by TAIR's user focus group and will be released to the public in May of 2000. The first version of our database and interfaces will integrate sequence, gene, genetic marker, polymorphism, and clone data. Effort will be dedicated to accurately attributing data to their contributors via links to publications and community data. The first version of the database will be released simultaneously with the MapViewer. During the second phase of development, we will be enhancing the database to include stocks, gene expression, and mutant phenotype data. In support of this, we will be developing controlled vocabulary terms to describe anatomical parts, developmental stages, environmental conditions, and plant-specific processes. Concurrently, the MapViewer will be enhanced to show sequence annotation of the completely sequenced chromosomes with details down to the nucleotide level. These two phases will establish the basis of our database and we intend to iterate the development process in the next few years to accommodate the rapidly growing and changing information, technology, and needs in genomic and post-genomic research.

55 Structural analysis of the Arabidopsis thaliana genome at Kazusa DNA Research Institute

Shusei Sato, Yasukazu Nakamura, Erika Asamizu, Takakazu Kaneko, Tomohiko Katoh and Satoshi Tabata

Kazusa DNA Research Institute, Kisarazu, Chiba 292-0812, JAPAN

In order to investigate the whole genetic system in higher plants, we have been operating 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 chromosomes 3 and 5 according to the international agreement of the Arabidopsis Genome Initiative (AGI). As of April 2000, sequence analysis of both chromosomes 3 and 5 is nearing completion. We have already made available 24.5 Mb of non-redundant finished sequences through the public database and our web database KAOS (Kazusa Arabidopsis data Opening Site) at <http://www.kazusa.or.jp/kaos/>. In KAOS, we also developed a section named Arabidopsis Genome Displayer (AGD) which enables users to browse original annotation and re-computational information for all AGI-derived sequences. As of April 2000, AGD contains genomic information of 104.4 Mb regions and 95.8 Mb of them are ready to browse. To complement the currently available cDNA

information and to facilitate the gene modeling process, we have been conducting large scale cDNA sequencing project using normalized libraries and size selected (3 kb and longer) libraries from various sources. A total of 13925 5' and 38690 3' ESTs from normalized and the size selected libraries of aboveground organs, roots, flower buds and siliques have been accumulated. The 3' ESTs were grouped into 11900 independent species, of which 44 % did not have matched sequences in the registered Arabidopsis ESTs. These data will be released through the public database and our web database in June 2000. The latest status of our projects will be presented.

56 Microarray analysis of Arabidopsis circadian gene expression patterns
Robert Schaffer, Jeff Landgraf, Verna Simon, Monica Accerbi, Matt Larson, Pamela Green, Ellen Wisman

MSU-DOE Plant research Laboratories, MSU, East Lansing, MI. 48824 USA

Circadian regulation of gene expression is common in higher organisms, it is controlled by an internal clock with an approximate 24 hour period which allows the anticipation of the coming day. Plants show a number of circadian regulated effects including leaf movement, flowering time and gene expression. cDNA microarrays were applied to identify global gene expression changes in response to light dark cycling conditions. We generated Arabidopsis microarrays containing 11,000 non redundant ESTs, and used these to measure the types of rhythms seen firstly in day night cycling conditions, and secondly under continuous light and dark. By repeating hybridisations we validated these results and using clustering analysis we are able to identify groups of genes with distinct cycling patterns. In these patterns novel genes could be grouped with already characterised cycling genes, for example *lhy* and *ccr2*. Of the 11,000 EST clones only 1% were strongly circadianly regulated. The changes in cycling gene expression was also investigated in the *lhy* mutant, where a myb transcription factor is constitutively expressed, causing all circadian controlled phenotypes become arrhythmic. This work is supported by the NSF.

57 Activities of the Arabidopsis Biological Resource Center in 1999-2000
Randy Scholl, Doreen Ware, Deborah Crist, Emma Knee, Luz Rivero, Jeff Cotrill, Nasser Assem* and Sakti Pramanik*

Dept. of Plant Biology, Ohio State Univ. and *Dept. of Computer Science and Engineering, 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 publishes stock information in the AIMS database and the ABRC Web Catalog (both found at <http://aims.cse.msu.edu/aims/>). The complete Web catalog can be downloaded as a PDF file from the Web site. A number of new stocks have been added to our collections in the past year, including: A) More than 170 new mutant stocks. B) Pools of T-DNA lines representing more than 40,000 new lines so that the numbers available and to be released by summer, 2000 will exceed 100,000, C) New clone accessions, D) New libraries, E) Additional ESTs, and F) The T-DNA population from Drs. Sussman and Amasino, from which seed samples are used to isolate knockout lines identified by the AFGC knockout facility (AKF) as containing specific insertions. During the past year, ABRC distributed 45,000 seed and 23,000 DNA stocks to

researchers worldwide, and stock usage continues to be very high. A project to isolate additional DNAs from T-DNA populations has been initiated, and some of these DNAs should be ready for distribution in the near future. ABRC is supported by the National Science Foundation.

58 A cosmid contig completes the physical map on the lower arm of Chromosome 3
Kathrin Schrick and Gerd Jürgens

ZMBP, University of Tübingen, D-72076 Tübingen, Germany

The closing of gaps in the physical map is a crucial step in the completion of the Arabidopsis genome sequence. In the process of cloning the FACKEL (FK) gene using a map-based approach, we established contigs to build a physical map on the bottom of Chromosome 3. The FK gene was mapped south of the RFLP marker mi456. Additional CAPS and RFLP markers corresponding to this region were developed, and genetic data describing the recombination frequency in this region were generated. Using IGF BAC (Mozo et al., 1998) and pBIC20 cosmid (Meyer et al., 1994) libraries, a BAC contig comprised of 14 BACs spanning 300 kb and a corresponding cosmid contig spanning 130 kb were established to cover the region containing the gene. Eighteen overlapping cosmids were transformed to identify the FK gene by complementation. The cosmid contig established during this work completes the physical map between BACs F8J2 and F6C4 on chromosome 3 (http://www.arabidopsis.org/cgi-bin/maps/Pmap?contig=5S_rDNA-F28P10-Sp6&clone=F8J2).

Meyer, K.M., P. Leube, and E. Grill. 1994. A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. *Science* 264: 1452-1455.

Mozo, T., S. Fischer, S. Meier-Ewert, H. Lehrach, and T. Altmann. 1998. Use of the IGF BAC library for physical mapping of the *Arabidopsis thaliana* genome. *Plant J.* 16: 377-384.

59 Construction of Arabidopsis Full-length cDNA Libraries by Biotinylated CAP Trapper and Monitoring Gene Expression Pattern Under Dehydration and Cold Stress using Full-length cDNA Microarray

Seki, M.1,2, Narusaka, M.2, Abe, H.3, Yamaguchi-Shinozaki, K.3, Carninci, P.4, Hayashizaki, Y.4 and Shinozaki, K.1,2

1Lab. Plant Mol. Biol., RIKEN, 2Plant Mut. Expl. Team, Plant Fun. Gen. Res. Gr., GSC, RIKEN, 3Biol. Res. Div. JIRCAS, 4Genome Sci. Lab. RIKEN

Full-length cDNAs are essential for functional analysis of plant genes. We constructed Arabidopsis full-length cDNA libraries 1) from plants in different conditions, such as dehydration-treated plants and cold-treated plants, and plants at various developmental stages from germination to mature seeds. We used 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. Until now, we have done single-pass sequencing of 13,109 cDNA clones from the 5' end.

These clones were classified into 6,345 independent genes. We also prepared a Arabidopsis full-length cDNA microarray using ca. 1300 full-length cDNAs including drought-inducible genes. The cDNA microarray was used to identify drought- and cold-inducible genes, and target genes of DREB1A, a transcription factor that controls stress-

inducible gene expression. The rd29A, cor15a, kin1, kin2, rd17, and erd10 genes were also identified as DREB1A target genes using the cDNA microarray, which confirmed the previous report. Also, 6 genes, such as a homolog of wheat putative cold acclimation protein, were identified as novel drought- and cold- inducible genes, and DREB1A target genes. These results show that our full-length cDNA microarray is a useful material to analyze expression pattern of Arabidopsis genes under dehydration and cold stress and to identify target genes of stress-related transcription factors. 1) Seki, M., Carninci, P., Nishiyama, Y., Hayashizaki, Y., and Shinozaki, K. (1998) Plant J. 15: 707-720.

60 Genome-Wide Survey of Receptor-Like Kinases in Arabidopsis thaliana
Shinhan Shiu and Anthony B. Bleecker

Department of Botany, University of Wisconsin-Madison

Receptor-like kinases (RLKs) in plants involves in various processes ranging from developmental regulation to disease resistance. RLKs usually have a three-domain organization: an extracellular domain, a transmembrane region, and an intracellular kinase domain. In Arabidopsis, a family of several hundred RLKs has been sequenced that represent a significant fraction of coding sequences in the Arabidopsis genome. Based on the predicted extracellular domain sequences, these receptors can be categorized into 4 major and several minor subfamilies. However, it is not known if this classification reflects functional or evolutionary relationship among subfamily members. In order to address this question, We conducted multiple alignments using the full length, the kinase domain, or the extracellular domain sequences of RLK proteins. Together with the information on the intron/exon structure of RLK genes and their location on the chromosomes, we attempt to reconstruct the evolutionary history for some of the family members and to use this information as a basis for the analysis of RLK function via knockout screen. In particular, we are in the process of generating triple mutants of a subset of three related RLKs, TMK1, 2, and 3. The implications of the alignments and the current progress in generating the mutant will be presented.

This work is supported by USDA(98-35301-7674).

61 Whole-Chromosome Gene Expression Analysis in Arabidopsis thaliana
Erik Snesrud, Baoping Zhao, Christopher D. Town, and John Quackenbush
The Institute for Genomic Research

Genomics has produced a revolution in plant biology and genome sequencing projects in plants are progressing rapidly. While the sequencing of the Arabidopsis thaliana genome will be completed within months, the identification and annotation of the genes and the role they play in development, disease, and response to environmental stress remains a significant challenge. In Arabidopsis, there are an estimated 30,000 genes, but fewer than 1,500 have annotated, full-length coding sequences in GenBank and the nearly 40,000 EST sequences represent only a fraction of total gene content; nearly three quarters of the annotated genes have no direct experimental support.

Using the gene predictions for the completed sequence of Arabidopsis chromosome II as a model, we have begun a program to validate the gene predictions using cDNA microarrays. We have designed primers and amplified the 3' ends of the 4,442 predicted genes on the chromosome and constructed microarrays containing those genes. These

arrays are being used to assess patterns of gene expression in a variety of plant tissues and developmental stages in order to provide experimental validation for the gene predictions and to develop an expression map of the chromosome. In addition, the arrays contain genomic sequencing clones from a minimal tiling path spanning a 1.5 Mb region that will allow us to assess the frequency with which genes were missed in the initial annotation and may provide additional information on the accuracy of the gene models.

62 Endogenous targets of transcriptional gene silencing in *Arabidopsis thaliana*
Andrea Steimer¹, Paolo Amedeo¹, Karin Afsar¹, Paul Fransz², Ortrun Mittelsten
Scheid¹, Jerzy Paszkowski¹

¹Friedrich Miescher Institute, PO Box 2543, 4002 Basel, Switzerland

²Institute of Plant Genetics and Crop Plant Research, Correnstrasse 3, 06466
Gatersleben, Germany

Transcriptional gene silencing (TGS) frequently inactivates foreign genes integrated into plant genomes but very likely also suppresses an unknown subset of chromosomal information. Accordingly, RNA analysis of mutants impaired in silencing should uncover endogenous targets of this epigenetic regulation. We compared transcripts from wild-type *Arabidopsis* carrying a silent transgene with RNA from the isogenic transgene-expressing TGS mutant *mom1* (Amedeo et al., 2000). Two major classes of related cDNA clones, termed TSI for transcriptionally silent information, were identified representing endogenous RNA expressed only in the mutant. Corresponding RNAs were also found in all other mutants affected in TGS regardless whether their genomic templates maintained DNA methylation (*mom1*, Amedeo et al., 2000; *sil1*, Furner et al., 1998) or became hypomethylated (*ddm1*, Vongs et al., 1993; *som1* to *som8*, Mittelsten Scheid et al., 1998). This implies that TGS in general and not a particular mutation controls transcriptional activity of the TSI templates. TSI is encoded by repeats present in heterochromatic pericentromeric regions of *Arabidopsis* chromosomes. These repeats share sequence homology with the 3' terminal part of the putative retrotransposon *Athila* (Pélissier et al., 1995). However, the transcriptional activation does not include the transposon itself and does not promote its movement. Thus, foreign genes in plants also encounter the epigenetic control normally directed, at least in part, towards a subset of pericentromeric repeats.

Amedeo, P., Habu, Y., Afsar, K., Mittelsten Scheid, O., and Paszkowski J., *Nature*, in press

Furner, I. J., Sheikh, M. A., and Collett, C. E. (1998). *Genetics* 149, 651-662.

Mittelsten Scheid, O., Afsar, K., and Paszkowski, J. (1998). *Proc. Natl. Acad. Sci. USA* 95, 632-637.

Pélissier, T., Tutois, S., Deragon, J. M., Tourmente, S., Genestier, S., and Picard, G. (1995). *Plant Mol. Biol.* 29, 441-452.

Vongs, A., Kakutani, T., Martienssen, R. A., and Richards, E. J. (1993). *Science* 260, 1926-1928.

63 High throughput screening for *Arabidopsis* knock-out mutants using microarrays
Sabine Steiner-Lange, Koen Dekker and Heinz Saedler

ZIGIA, Max Planck Institute for Plant Breeding, Carl-von-Linné-Weg 10, 50829 Koeln, Germany

The ZIGIA project at the Max Planck Institute for Plant Breeding in Cologne focuses on functional genomics in Arabidopsis. One goal is the systematic characterisation of all lines of our Arabidopsis knock-out mutant population, which is mutagenised by insertions of the maize transposon En/Spm. It consists of 11 000 lines each carrying 5 to 20 copies of the transposon. The population has been used successfully for mutant characterisation following both forward and reverse genetic approaches. Since July 1998, screens for ca. 380 genes on DNA-pools have been performed to identify knock-out mutants. In order to increase the throughput of reverse genetics and to enable a systematic characterisation of the En-integration sites of all individuals, a new method using microarrays was established.

For the production of the microarrays DNA is isolated from each plant individually. The isolated DNA is then cleaved with two different restriction enzymes followed by ligation of linkers to DNA ends. Two successive rounds of PCR using transposon- and linker-specific primers allow the amplification of the transposon flanking regions. Then these mixtures of amplified flanking regions, each derived from a single plant, are spotted on nylon membranes. By single hybridisations using a gene specific probe, candidate mutant plants can be identified.

Filters representing 2880 plants (and thus about 30 000 insertions) have been utilized for mutant identification. Control experiments using probes each corresponding to a known transposon-integration site in one of the plants represented on the filter, showed that about 70 % of existing insertions can be identified. When screening for knock-out mutants of six genes, for which no mutant had been discovered before, candidate plants were found in five cases. This shows that even filters representing less than a third of the plants of our population can provide a useful tool for the identification of mutant plants. Filters with material for all 11 000 plants are expected to be available by the end of the year. This will allow plant scientists to identify individual knock-out mutants in Arabidopsis through just one hybridisation using a gene specific probe.

64 Graphical display tools created for Arabidopsis microarray data
Sterky, F., Gollub, J., Finkelstein, D.B., Sherlock, G., Somerville, S., Cherry, J.M.
Carnegie Institution of Washington, Dept. of Plant Biology, Stanford

The Arabidopsis Functional Genomics Consortium (AFGC) is now in full production, generating microarray data for the plant scientific community. Data produced at AFGC are stored within the Stanford Microarray Database (SMD). Raw data, scanner results and biological information for these experiments (and others) are accessed via the web (afgc.stanford.edu/SMD/). AFGC has begun to explore some special data analysis interests. We have developed tools to enhance the graphical display of microarray data from SMD. The AFGC experiments will in most cases be performed as duplicates where the dyes have been reversed for the two channels (i.e. reversed duplicates). These duplicates will help in accessing the confidence in conclusions drawn from the experiment. For example, software has been developed to plot the logarithm of the ratio (channel 2 over channel 1) for the two experiments, which will give an overview of the quality of the experiments. Other tools allow the user to customize their own plot using intensity or ratio values for a selected experiment or to follow the 'history of behavior' of

a selected gene through several experiments. These tools enable detailed studies of specific groups of spots or genes. For more detailed analysis there are other features at the SMD site, such as the hierarchical clustering. It is our intention to allow the graphics to become a visual alternative for selection of interesting genes for further analysis. That is genes or sets of genes will be selectable from the graphical images.

65 Epigenetic States in the Arabidopsis Genome

Trevor L. Stokes and Eric J. Richards

Washington University

The modification and packaging of DNA can affect how genetic information is interpreted. Previously reported *ddm1* mutants potentially connect DNA methylation to chromatin dynamics. DDM1 encodes a gene homologous to a class of energy dependent chromatin remodelling factors, SWI2/SNF2 and loss of DDM1 produces a DNA hypomethylation phenotype. We have used *ddm1* mutants to better understand the function of DNA methylation in genomic stability. Previously, a dwarfing variant, *bal*, was generated in a *ddm1* hypomethylation background and is stable in a DDM1 background. Despite segregating as a single Mendelian trait, the *bal* phenotype can be reverted at a high frequency (>5 percent) in response to chemical or physical mutagenesis. The metastability of *bal* suggests that *bal* represents a novel epigenetic state at the BAL locus. In an effort to better understand the molecular events underlying the *bal* phenotype, we have taken a map-based cloning strategy. We have delimited a genetic window that translates to a physical window of 150 Kb. There are two notable features in this region, a cluster of disease resistance genes interspersed by two retroelements. By examining the steady state RNA levels, we detect a 6.5 Kb message originating from the region the amount of which corresponds well with the severity of the *bal* phenotype. The corresponding message is poly-adenylated, contains intronic sequence and predominantly originates from one coding region in the gene cluster.

66 Arabidopsis thaliana transcription factor macroarray for gene expression analysis

Ralf Stracke, Carla Schoenbohm, Martin Werber, Imre E. Somssich and Bernd Weisshaar
Max-Planck-Institute for Plant Breeding Research

Plant transcription factors are central components of the regulation of many processes in plant biology, including cell development, differentiation, cell cycle, secondary metabolism, disease resistance and defence responses. Specific transcription factor families such as MYB domain proteins (helix-turn-helix domain), bZIP proteins (basic region/leucine zipper domain), WRKY proteins (Zink finger domain with a Cx4-7Cx22-23HxH/C pattern), bHLH proteins (basic region/helix-loop-helix domain) are known to exist in plants. For the majority of these genes, however, little functional information is available.

Current research in many labs is aiming at the functional dissection of the role of a wide variety of plant transcription factors by means of molecular and genetic approaches. To this end, we generated a transcription factor cDNA macroarray for parallel expression monitoring. This TF array contains about 200 PCR products specific for R2R3- and R1R2R3-MYB, WRKY, and bHLH gene family members. Additionally, it carries a set of 45 target genes with known regulatory characteristics which serve as controls. For quantification and standardization of hybridization signals several constitutively

expressed genes were included, as well as a synthetic cDNA fragment detecting an artificial mRNA with which the probe will be "spiked". For simple chip production, gene-specific cDNA regions were PCR amplified, cloned into the vector pCR2.1-TOPO and the respective inserts were amplified with vector primers. Resulting PCR products were spotted in grids as duplicates on filter membranes with a robot (BioRobotics). The spotting efficiency was controlled by oligo hybridization using a common vector sequence as target. We will present results from first experiments using probes from *A. thaliana* cells irradiated with UV-light. A combination of commercially available and self-written software/scripts will be used for data evaluation.

67 Heat-shock tagging: a simple method for expression and isolation of plant genome DNA flanked by T-DNA insertions

Taku Takahashi, Fumito Jingu, Shio Matsuhara and Yoshibumi Komeda

Division of Biological Sciences, Graduate School of Science, Hokkaido University, N10, W8, Sapporo 060-0810, Japan

The notion underlying our 'heat-shock tagging' strategy (Matsuhara et al., 2000. *Plant J.* 22: 79-86) is that, in addition to a simple insertional mutagenesis, a T-DNA construct carrying a heat-shock promoter toward plant genome DNA could induce the transcriptional read-through by which sense or antisense transcripts are synthesized unidirectionally according to the orientation of the T-DNA insertion, and that its expression can be manipulated by heat-shock treatment of plants. Furthermore, unlike usual labored cloning methods (plasmid rescue, tail-PCR, and IPCR), the flanking genome region can be easily and selectively identified from heat-shocked plants as a cDNA fragment by RT-PCR using an oligo(dT) primer and a T-DNA-specific primer, only when tagged transcripts are polyadenylated. We performed RT-PCR experiments by using only 12 RNA samples, each of which represented a mixture of 10 independent transformants, and so far identified 13 insertions into the correct orientation of various genes. We also cloned cDNA fragments derived from antisense transcripts and intergenic spacer regions but less frequently, suggestive of a higher efficiency of polyadenylation by true poly(A) signals. Our results suggest that the heat-shock tagging is one of the most effective strategies for identifying gene disruption and will be a powerful tool for saturation mutagenesis in the post-sequencing era.

68 The Arabidopsis Genome Sequencing Project at TIGR

C. Town, S. Kaul, X. Lin, M-I. Benito, R. Buell, C. Ronning, A. Walts, J. Jiang², J. Heslop-Harrison³, D. Preuss⁴, J. Eisen, O. White, S. Salzberg, C. Fraser, C. Venter⁵
The Institute for Genomic Research, Rockville, MD, USA; ² Univ. of Wisconsin, Madison, WI, USA; ³ John Innes Centre, Norwich, UK; ⁴ Univ. of Chicago, Chicago, IL, USA; ⁵ Celera Genomics, Rockville, MD, USA

At TIGR, we have completed the sequence of chromosome 2 of *Arabidopsis thaliana* as two gap-free assemblies (contigs) of 3.6 Mb and 16 Mb, respectively. Chromosome 2 represents 15% of the genome and encodes 4,037 genes, 48% of which have no predicted function. Approximately 250 tandem gene duplications were found. In addition, large-scale duplications of approximately 0.5 and 4.5 Mb between chromosomes 2 and 1 and between chromosomes 2 and 4, respectively were detected. Sequencing of nearly 2 Mb within the genetically defined centromere revealed a low density of recognizable genes

and a high density and diverse range of degenerate and presumably inactive mobile elements. With the completion of chromosome 2, we have started sequencing regions of chromosomes 1 and 3, in conjunction with other groups in the Arabidopsis Genome Initiative. In parallel with the high throughput sequencing, additional work is aimed at a more complete analysis of the Arabidopsis genome. We discovered a large fraction of an intact mitochondrial genome near the centromere of chromosome 2. We are currently exploring the evolutionary history of this insertion by analysis of other ecotypes as well as by sequencing the mitochondrial genome from Columbia. Other work is aimed at integrating orphan and unanchored contigs into the genome. Fiber-FISH is being used to investigate the relationship between isolated contigs near CEN3, and sample sequencing is being used to determine whether totally unanchored contigs are in fact part of the Arabidopsis genome. Supported by NSF, USDA and DOE.

69 Jumbled genes: T-DNA-associated chromosomal rearrangements, & implications for genomics & reverse-genetics

Daniel M. Vernon¹ & Frans Tax²

¹Biology Dept., Whitman College, Walla Walla, WA 99362. ²MCB Dept., University of Arizona, Tucson, AZ 85721

T-DNA insertion mutations are a valuable resource for studies of gene function in Arabidopsis, serving as the basis for both forward and reverse genetic strategies and as a source of sequence tags from large collections of mutant lines. We have identified internal chromosomal duplication/rearrangements in 2 of 8 T-DNA insertion lines characterized in detail by our laboratories. These rearrangements were identified in the course of both reverse- and forward-genetics projects. Both were directly associated with simple T-DNA inserts that exhibited normal Mendelian segregation, and both were detected only through a combination of detailed molecular analysis and mapping of the kan-resistance phenotype imparted by the T-DNA. In one study, we characterized the embryo-defective^{emb88} mutation. Although ^{emb88} has been mapped genetically to chromosome I, molecular analysis of genomic DNA directly adjacent to the T-DNA left border revealed sequence from chromosome 5. SSLP mapping of the T-DNA confirmed that a >40 kBP region of chromosome 5 had inserted with the T-DNA into the ^{emb88} locus on chromosome I. A similar scenario was observed in a reverse-genetic study of the LR-RPK gene. A prospective insertion mutation was identified by PCR screening of T-DNA lines. Whereas wild-type LR-RPK is encoded on lower chromosome 4, mapping of the T-DNA localized the mutant allele to chromosome 5. In both these cases, sequence of T-DNA flanking regions did not provide an accurate picture of DNA disruption, because flanking sequences had apparently duplicated and inserted into other loci in different chromosomes. Our results indicate that T-DNA insertion lines- even those that exhibit straightforward genetic behavior with regard to T-DNA and developmental phenotypes- may contain an unexpectedly high frequency of duplication/transpositions. Such rearrangements can interfere with reverse-genetic analyses and provide misleading information on the molecular basis of mutant phenotypes. Thus, meaningful interpretation of phenotypes or sequence information from T-DNA mutants requires genetic mapping of T-DNA insertion sites, in addition to standard genetic and molecular characterization of mutant alleles. -Supported by NSF [DV] & USDA [FT]

70 Gene expression profiling in Arabidopsis using DNA microarrays
Ellen Wisman, Jeff Landgraf, Robert Schaffer, Monica Accerbi, Vernadette Simon, Matt Larson and Pam Green

MSU-DOE Plant Research Laboratory, East Lansing, MI 48854, 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. In the first year of our project we have printed and tested a microarray containing 11,000 non-redundant Arabidopsis ESTs selected from the 37,000 ESTs available in the public domain. The DNA fragments spotted on the microarray have been amplified from the collection of EST clones sequenced by Tom Newman. By hybridizing these arrays with probes corresponding to mRNA extracted from different tissues global gene expression patterns can be investigated. To this end, the labeling and hybridization techniques have been optimized. The microarray facility has started its service at the beginning of this year. In the first of three rounds of applications we have received 44 proposals from scientists of 6 different countries. Most experiments compare mutant to wild type or treated to untreated plants. Two experiments use Brassica plant material rather than Arabidopsis. The proposal titles and our services can be viewed on the website <http://afgc.stanford.edu/>. Our project will determine the gene expression patterns in different tissues and organs or from Arabidopsis plants subjected to different stimuli. We will also examine the expression of plant specific genes of unknown function under a set of standardized conditions.

Contact Address: Ellen Wisman wismanp@pilot.msu.edu

71 GLOBAL GENE EXPRESSION PATTERN OF ARABIDOPSIS THALIANA BY A HIGH-DENSITY OLIGONUCLEOTIDE PROBE ARRAY

Tong Zhu, Hur-Song Chang, Bin Han, Guangzhou Zou, Yen Kim Tran, and Xun Wang
Novartis Agricultural Discovery Institute, Inc., San Diego, CA 92121, USA

As a model system, Arabidopsis thaliana has been widely used in various aspects of biological studies, such as genomics, genetics, and developmental and molecular biology. In order to reveal the molecular events and their regulation of this unique organism involved in its development and responses to genetic and environmental changes, in collaboration with Affymetrix, we designed and used a high-density oligonucleotide microarray (GeneChip™) to profile its global gene expression patterns. This Arabidopsis oligonucleotide microarray consists of probes from 8775 Arabidopsis genes, which covers approximately one third of the genome. Each gene has 16 probe pairs including 16 perfect match and 16 mismatch probes. A total of ten duplicate experiments revealed the high reproducibility of the microarray, indicated by the average of 0.2% false positives. Using this microarray, global transcription profiles of Arabidopsis thaliana in various developmental stages, and their responses to different environments were generated and archived. Challenges and opportunities in microarray data production and mining will be presented.

72 Methylation signaling in an endogenous gene family

Lisa Barteo and Judith Bender

Johns Hopkins School of Public Health

We are using the endogenous phosphoribosylanthranilate isomerase (PAI) genes in the *Arabidopsis* ecotype WS to study the mechanism by which methylation is targeted to PAI sequences. In WS, there are four PAI genes present at three unlinked loci: PAI1 and PAI4 arranged as a tail-to-tail inverted repeat, a singlet PAI2 gene, and a singlet PAI3 gene. All four PAI genes are highly identical and heavily cytosine methylated over their regions of identity. In previous work, we showed that the PAI1-PAI4 inverted repeat produces a signal that can trigger methylation of PAI sequences, but the mechanism of this methylation signaling is unknown. Since methylation is found only over regions of PAI identity, it is likely that nucleic acid pairing at a DNA/DNA level and/or RNA/DNA level is involved. We have developed a genetic screen for mutants with decreased PAI methylation (dpm). Characterization of mutants isolated in this screen will allow us to elucidate the signaling mechanism used by the inverted repeat to target methylation to the other PAI genes. Using this screen, we have isolated a novel mutant, dpm1. Unlike previously isolated ddm methylation mutants, dpm1 does not cause a global decrease in genomic methylation. The characterization and mapping of the dpm1 mutation will be discussed.

73 Evidence of selective pressure at the RPP13 disease resistance locus of *Arabidopsis thaliana*

Peter Bittner-Eddy and Jim Beynon

Horticulture Research International, Wellesbourne, Warwickshire, CV35 9EF, UK

We are interested in the organisation, structure, and evolution of disease resistance genes in *Arabidopsis thaliana* that recognise the biotrophic oomycete, *Peronospora parasitica* (RPP genes). We have cloned functional RPP13 alleles from two *Arabidopsis* accessions (Nd-1 and Rld-2) that confer different resistance specificities and have compared them to the susceptibility allele from the Col accession [Bittner-Eddy et al. (2000), *Plant J.* 21, 177-188]. Comparison of the three RPP13 alleles revealed a higher rate of DNA divergence within the LRR domain, 78 to 82% identity overall, compared to the remainder of the gene (98-99% identity). In addition, out of 9 indels, 8 occurred within the LRR domain. We have now extended our analysis by identifying and sequencing two paralogs (T240 and T260) from Col, Nd-1, and Rld-2 that lie linked together 73 kb from RPP13. Unlike other R gene families, the three genes share only a moderate level of DNA homology (56-69% identity overall). Similar to RPP13, DNA identity of the non-LRR region ranged from 96-100% for the two paralogs across the three accessions. However, in contrast to the high intra-specific DNA divergence within the RPP13 LRR domain, we observed a high level of intra-specific DNA conservation among the LRR domains of T240 and T260 (93-100% identity). Furthermore, in an analysis of nucleotide substitutions we found evidence of positive selection ($K_a:K_s$ ratios 1) for amino acid diversification only within the RPP13 LRR domain. Taken together, this evidence strongly suggests that RPP13, unlike the two paralogs, has been or still is under strong evolutionary selection. The significance of this observation is underscored by the fact that the related species *Brassica oleracea* has T240 and T260 orthologs but appears to lack

RPP13 orthologs, indicating that the generation of RPP13 post-dates speciation of Arabidopsis and Brassica.

74 Natural Variation in Light Response

Borevitz, J.O.; Maloof, J; Dabi, T; Lutes, J; Weigel, D; Chory, J

Plant Biology Salk Institute 10010 N Torrey Pines Rd La Jolla CA 92037 USA

Since light is a major environmental component influencing plant development, natural populations must adapt to their unique light environment. To identify genes responsible for adaptation we have begun by surveying a large collection of Arabidopsis ecotypes. Hypocotyl length measurements were made on over 130 accessions under 5 light conditions- white, blue, red, far-red light, or in the dark, and 3 hormone conditions - GA, BRZ (an inhibitor of brassinosteroid biosynthesis), and ethylene. Wide genetic variation was seen in all conditions surveyed, confirming that natural populations of Arabidopsis are a great tool to understand the complex genetics of plant light response. While responses of some ecotypes varied across multiple conditions, other ecotypes had changes characteristic of known mutations. Data describing a natural loss of function phyA allele will be presented. Cluster analysis was used to create a phenotypic phylogenetic tree based on hypocotyl length and to compare how light and hormone conditions were related. Response to blue light, far-red light and BRZ were correlated suggesting that the signaling pathways share common components. Recombinant Inbred lines are being made from several ecotypes with unique light responses to subsequently map the genes responsible for quantitative variation in light response.

The Cape Verde Island/ Landsberg recombinant inbred line population was used to map Quantitative Trait Loci (QTLs) in 5 light and 2 hormone environments. One QTL on Chromosome 1 (24-27cM) may be 2 linked genes the first having an effect in blue and far-red light, and the 2nd having an effect in the white light, red light, and GA environments. Period length and flowering time experiments done by others also detect major and minor QTLs at this novel locus. A QTL was detected in white and red light at the PHYB locus, implicating PHYB as a candidate gene. A QTL at the ERECTA locus was detected in the BRZ, blue, and far-red environments, perhaps uncovering previously unknown pleiotropic effects of the erecta mutation. The genotype X environment interaction detected illustrates how QTL mapping can be used to identify pleiotropy and thus to determine how environmental responses are coregulated. The QTLs at Chromosome 1 (24-27cM), ERECTA and PHYB have been confirmed in near isogenic lines. Sequence variation at the PHYB locus in 12 ecotypes has been investigated for evidence of selection.

75 The cloning of a flowering time QTL reveals a novel allele of CRY2

Salah EL-Din EL-Assal (1), Carlos Alonso-Blanco (2), Anton J.M. Peeters (3) and Maarten Koornneef (1)

(1) Lab. of Genetics, Wageningen Univ., Dreijenlaan 2, 6703 HA Wageningen, The Netherlands; (2) Centro Nacional de Biotecnología, Campus Universidad Autónoma, 28049-Madrid, Spain; (3) Dept. of Plant Ecophysiology, Utrecht Univ., Utrecht, The Netherlands

In addition to induced mutants, genetic variation for flowering time is also found among natural populations (accessions or ecotypes). We analyzed the genetics of flowering time

in a recombinant inbred line (RIL) population derived from crosses between Landsberg erecta (Ler) and the accession Cape Verde Islands (Cvi) under three environments differing in day-length and/or vernalization treatment. A major flowering time locus, located at the top of chromosome 1, designated early day-length insensitive (EDI), was identified along with three other quantitative trait loci (QTLs) (Alonso-Blanco et al, 1998, Genetics 149:749-764). To characterize EDI, a near isogenic line was constructed by introgressing the Cvi allele (EDI) into the Ler background. This allele leads to earliness under both long- and short-day photoperiods, rendering Ler plants almost day-length neutral. To determine the precise map location for EDI, a segregating population was developed and genotyped with molecular markers. The segregation ratio fitted a single Mendelian gene at which the early flowering Cvi allele is dominant in relation to the late flowering Ler allele. In addition, an absolute co-segregation of EDI with the CRY2 gene was observed. We confirmed that EDI is CRY2 by transforming a CRY2-Cvi genomic fragment into Ler and found several transformants that exhibited the EDI flowering time behavior. Sequence analysis of the CRY2-Cvi and CRY2-Ler alleles revealed a candidate point mutation, located in the binding site of the flavin chromophore. Swapping experiments of fragments of the Ler and Cvi CRY2 sequences are underway and may prove if the candidate mutation is responsible for the phenotypic effect of this allele. Northern-blot analysis revealed that there is no difference in expression level between EDI and Ler. This suggests that differences in either protein stability or the interaction of CRY2 with target genes may explain why this specific CRY2 allele leads to early flowering in long- and short days.

76 Linkage disequilibrium and haplotype structure in the chromosomal region containing the flowering time locus FRI

Jenny Hagenblad and Magnus Nordborg

Department of Genetics, Lund University, Lund, Sweden

Linkage disequilibrium (or LD) has recently received a great deal of attention, primarily because of the potential utility of population associations for fine-scale mapping of human disease loci, but also because of the increasing use of haplotype data for historical or evolutionary inference. Regardless of the application, the extent of LD is of central importance. In the context of mapping, it determines how densely spaced the markers need to be, for example.

It is clear that LD should be much more extensive in partially selfing organisms like *Arabidopsis*. Intuitively speaking, because individuals will tend to be homozygous, recombination will be much less effective in creating new haplotypes.

We have investigated LD in *Arabidopsis* by sequencing a dozen 0.5-1 kb segments in 20 accessions from a worldwide sample. The segments were intermittently spaced in a 450 kb region surrounding the flowering time locus FRI. The effects of inbreeding were immediate in that few recombination events could be detected within segments.

However, recombination could usually be detected between the segments, sometimes over distances as short as 10 kb. Among other things, this shows that there is no "phylogeny of ecotypes": as in other sexual organisms, genealogical relationships change rapidly as we move along the chromosomes.

In order to investigate the potential utility of LD mapping, the flowering time phenotype of the sequenced individuals was determined. No clear associations between the

phenotype and the marker genotypes were observed, indicating that the phenotypic variation was not due to alleles at FRI. However, direct investigation of the FRI alleles in the sampled individuals revealed that FRI was nevertheless involved, and that the lack of associations was due to lack of power caused by substantial allelic heterogeneity. The data suggest that LD mapping might be useful if allelic heterogeneity can be minimized by sampling from a more restricted geographic region. Finally, the LD surrounding the FRI alleles can be used to gain insight into the evolutionary history of these alleles.

77 Natural Variation and QTL mapping as Tools for Cloning Glucosinolate Biosynthetic and Regulatory Loci

Kliebenstein, D.J., Lambrix, V., Figuth, A., Pedersen, D., Kroymann, J and T. Mitchell-Olds

Max Planck Institute for Chemical Ecology, Carl Zeiss Promenade 10, 07745 Jena, Germany

Glucosinolates are naturally occurring amino acid derivatives that may have important plant defense roles in against numerous pests. To elucidate these functions we are cloning the genes responsible for the biosynthesis and regulation of this complex class of secondary metabolites. Natural variation present in different *Arabidopsis thaliana* ecotypes enables us to identify, map and clone both simple mendelian loci and more complex quantitative trait loci (QTL). Additionally, our development of a high-throughput protocol for the extraction and detection of 50 different glucosinolates via HPLC is allowing us to rapidly identify and clone these genes. An analysis of 35 ecotypes and 3 different recombinant inbred populations, identified 10 naturally varying mendelian loci and an additional 15-20 QTLs controlling the biosynthesis or regulation of glucosinolate accumulation. Utilizing the high-throughput HPLC procedure and six different ecotypes, we have map base cloned 5 different enzymes involved in aliphatic glucosinolate biosynthesis (see posters by V. Lambrix and J. Kroymann).

Currently, I am cloning of five different QTLs that control the accumulation of either methionine- or tryptophan-derived glucosinolates. There are candidate genes for one of the methionine- and three of the tryptophan-derived glucosinolate QTLs. The three QTLs for tryptophan-derived glucosinolate accumulation co-map with genes responsible for tryptophan biosynthesis, indicating that a major control point for glucosinolate accumulation may be within the production of the base amino acid. Therefore, QTL mapping may be a useful tool for pathway flux analysis in plants. In the future, modification of glucosinolates found in *Arabidopsis* will allow us to study the impact of specific glucosinolates on insect herbivory and other plant/pest interactions. Progress on the characterization of the candidate genes as well as further work on the other QTLs will be presented in this poster.

78 Towards the molecular identification of QTLs in *Arabidopsis*

Maarten Koornneef (1), Leonie Bentsink (1), Salah EL-Din EL-Assal(1), Anton J.M. Peeters (2), Carlos Alonso-Blanco (3)

(1) Lab. of Genetics, Wag Univ., Dreijenlaan 2, 6703 HA Wageningen, The Netherlands; (2) Dept. of Plant Ecophys., Utrecht Univ., Utrecht, The Netherlands. (3) Centro Nacional de Biotecnología, Campus Univ. Autónoma, Cantoblanco, 28049-Madrid, Spain

In addition to using induced mutants, genetic variation is also found among natural populations (accessions or ecotypes) of *Arabidopsis*. This variation is present especially for adaptive traits, which include flowering time, seed dormancy, stress tolerance, secondary metabolism etc. The analysis of this variation up to the molecular level requires an accurate mapping, using QTL procedures, the development of near isogenic lines, cloning and transformation experiments (Alonso-Blanco and Koornneef, *TPS* (2000) 5: 22-29. We demonstrated the feasibility of this approach in the analysis of a recombinant inbred line (RIL) population derived from crosses between *Landsberg erecta* (Ler) and the accession *Cape Verde Island* (Cvi). In this population we analyzed the genetics of seed and fruit size, flowering time, circadian period length, raffinose oligo saccharide content and seed dormancy. Further experiments aiming to the molecular identification of QTLs is focused on two large effect QTLs: 1) a major flowering time QTL designated early day -length insensitive (EDI), which has identified a novel allele of the *CRY2* gene; 2) a QTL affecting seed dormancy, named 'delay of germination' (DOG). The analysis of seed dormancy and flowering time demonstrates how such complex traits can be dissected into various components and are amenable to map based cloning.

79 Natural variation for glucosinolates controlled by a small gene family

J. Kroymann, S. Textor, D. Kliebenstein, S. Ring, A. Figuth, D. Schnabelrauch, T. Mitchell-Olds

Max-Planck-Institute of Chemical Ecology, Carl-Zeiss-Promenade 10, D-07745 Jena, Germany

Glucosinolates (mustard oil glucosides) constitute a major component of plant secondary metabolites in *Arabidopsis* and other Brassicaceae. Glucosinolates and their breakdown products (isothiocyanates, nitriles, thiocyanates, epithiocyanates, etc.) may contribute to plant defense against various pests, mediate feeding behavior of herbivores, and influence oviposition preferences of insects. They also determine quality and flavour of important crop plants such as mustard, canola, cauliflower or broccoli.

In *Arabidopsis*, more than 20 different glucosinolates have been identified. However, *Arabidopsis* ecotypes vary remarkably in composition and quantity of glucosinolates. A major genetic locus underlying this natural variation is *ELONG* on top of Chr. V (1, 2). We elucidate control of glucosinolates in the *ELONG* region. Our current model correctly predicts experimental results on glucosinolate biochemistry and inheritance.

1: Magrath, R., Bano, F., Morgner, M., Parkin, I., Sharpe, A., Lister, C., Dean, C., Turner, J., Lydiate, D., and Mithen, R. (1994) Genetics of aliphatic glucosinolates. I. Side chain elongation in *Brassica napus* and *Arabidopsis thaliana*. *Heredity*, 72, 290-299.

2: Campos, H., Magrath, R., McCallum, D., Kroymann, J., Schnabelrauch, D., Mitchell-Olds, T., and Mithen, R. (2000) β -Keto acid elongation and glucosinolate biosynthesis in *Arabidopsis thaliana*. *Theoretical and Applied Genetics*, in press.

80 Toward understanding of parental gene regulation in *Arabidopsis* allotetraploids

Hyeon-Se Lee and Z. Jeffrey Chen

Texas A&M University

Polyploidy, an important feature for plant evolution, is resulted from duplication of a complete set of whole genome (autopolyploid) or combination of two or more related but

different genomes (allopolyploid). It is estimated that over 70% of flowering plants, including important crops (e.g., canola, coffee, cotton, oats, soybean, sugarcane, and wheat), are polyploid. We are interested in understanding of how the expression of parental genes from different progenitors is regulated in plant allopolyploids. We hypothesize that plants use DNA sequence-independent or epigenetic mechanisms to regulate expression of redundant genes resulted from genome duplication. An extreme example is selective silencing of one parental set of rRNA genes in an interspecific hybrid or allotetraploid, a phenomenon known as nucleolar dominance. This silencing is enforced by chromatin modifications involving DNA methylation and histone deacetylation. We predict that other duplicate genes in addition to rRNA genes are subject to silencing during polyploid formation. Using AFLP-cDNA display method, we have investigated gene expression profiles in allotetraploid *Arabidopsis suecica* and its two progenitors, *A. thaliana* and *Cardaminopsis arenosa*. This method can distinguish between parental genes that are differentially expressed in the hybrids. Among ~1,000 genes analyzed, over 70% of parental genes are non-polymorphic and presumably co-expressed and 25% of genes are polymorphic with co-expression patterns in the allotetraploid. Interestingly, about 1-5% of them was identified as candidate genes that are differentially expressed in the allotetraploid. The silenced genes could be of either parental origin with a tendency of more silenced genes derived from *A. thaliana* than *C. arenosa*. After sequencing the cDNA fragments, we have identified at least five genes with known or unknown function. The role of epigenetic regulation in global control of gene expression during polyploid formation and evolution will be investigated and discussed.

81 Expression of a methylated gene family in *Arabidopsis*

Stacey Melquist and Judith Bender

Johns Hopkins University School of Public Health

We are using phosphoribosylanthranilate isomerase (PAI) gene family in *Arabidopsis* to study the molecular relationship between methylation and loss of gene expression. In the ecotype *Wassilewskija* (WS), there are four PAI genes, two of which are arranged as a tail-to-tail inverted repeat (PAI1-PAI4) and the other two arranged as single genes (PAI2, PAI3). PAI1, PAI2, and PAI4 are 99 percent identical over a region of 2 kb including upstream sequences, exons, introns, and downstream sequences. All PAI genes are heavily cytosine methylated over their regions of PAI identity starting at the homology boundary 350 bp upstream of the translational start and ending beyond the translational stop. Previous analysis of PAI gene expression in WS indicated that PAI1 is the only PAI gene expressed, whereas PAI2, PAI3, and PAI4 are silenced.

We are interested in determining what elements allow PAI1 to be expressed despite methylation. PAI1-PAI4 is a recent duplication from the progenitor PAI2 locus, and has a S15a ribosomal protein gene promoter fused 500 bp upstream of the PAI homology/methylation boundary, thereby creating a synthetic promoter. WS cDNA analysis indicates that at least some PAI1 transcripts initiate at the upstream S15a promoter. We hypothesize that PAI1 is uniquely expressed because the S15a transcription start site lies far enough upstream of the methylated region that transcription is not impeded. To test this hypothesis, we are using multi-copy transgenes containing PAI1 upstream sequences to promote a spread of methylation into the S15a promoter region of

the endogenous PAI1 gene. Preliminary results suggest that upstream methylation leads to silencing of PAI1 expression. Therefore, we are exploiting this method to map the boundary where methylation can extend relative to the transcription start site before transcription is impeded.

82 Natural Variation in Arabidopsis Seedling Photomorphogenesis: a Candidate Gene Approach

Alan Pepper and Ning Kang

Department of Biology, Texas A & M University

It has been suggested that non-neutral natural genetic variation present in plant populations is a potentially valuable source of alleles for the genetic study of responses to environment. Unlike alleles derived from traditional mutagenesis and screening experiments, natural variants have been 'filtered' by natural selection to eliminate alleles with pleiotropic effects. In theory, natural variation should be an excellent source of alleles in genes that act in specific environmental signaling pathways, or of alleles with effects in specific signaling pathways. We have examined natural variation in deetiolation responses (hypocotyl length) in a variety of light conditions (color and quantity) using a collection of single-seed descent populations derived from 12 Arabidopsis 'ecotypes' obtained in a variety of edaphic environments. Interecotypic genetic variation was observed in all light conditions examined. The genetic basis of ecotypic differentiation was examined for several ecotypes through crosses to ecotype Columbia (Col-0) and subsequent analysis of F1 and F2 progeny. In one case, differences in hypocotyl length appear to have been conditioned by alternate semi-dominant alleles at a single locus. Single-locus QTL analysis was performed to identify the location of the gene underlying this trait. To determine if this gene were novel or previously identified through classical 'forward genetics', we utilized several CAPS markers derived from cloned genes known to influence hypocotyl length (e.g. PHYA, PHYB, LHY1, CRY1, HY5, etc). QTL mapping results obtained using this 'candidate gene' approach will be presented.

83 Natural variation in genomic methylation levels

Nicole C. Riddle, Joshu Shih, and Eric J. Richards

Washington University, Department of Biology, St. Louis, MO 63130

Our lab is interested in gaining an understanding of the regulation of genomic 5-methylcytosine. We have previously reported the isolation of induced mutations that affect genomic methylation levels. Natural variation is another potential source for identifying modifier loci controlling genomic methylation. The methylation of the major ribosomal RNA gene clusters (rDNA) and centromeric 180 bp repeats in a variety of Arabidopsis ecotypes were surveyed by Southern analysis. We found that the ecotypes exhibited a wide range of different levels of rDNA methylation. Many ecotypes have levels of methylation similar to Columbia, while a few ecotypes such as Can-0 and Mt-0 show surprisingly low levels of rDNA methylation. Other ecotypes isolates appear to have more than one type of rDNA methylation patterns, and include individuals with both high and low levels of rDNA methylation. In these ecotypes, the rDNA methylation levels seem to be stably inherited from parent to offspring and do not appear to segregate. Experiments to determine the basis of this differential rDNA methylation are in progress, and results will be reported.

84 Phenotypic characterization of trichome distribution and molecular analysis of a candidate gene (GLABROUS1) in *Arabidopsis thaliana* and the close relative *Arabidopsis lyrata*

Schlötterer Christian¹, Harr Bettina¹ & Hauser Marie-Theres²

Institut für Tierzucht und Genetik, Veterinärmedizinische Universität Wien, Vienna, Austria¹; Center of Applied Genetics, University of Agricultural Sciences Vienna, AUSTRIA²

GLABROUS1 (GL1) belongs to the large family of MYB transcription factors and is known to play a central role in trichome initiation. We studied trichome distribution and the molecular variation in 26 *A. thaliana* accessions. Trichome density on rosette leaves was highly variable among them. On the molecular level, we detected substantial sequence variation in a 3 kb region which includes the complete coding sequence of the GL1 locus ($P = 0.01$). Phylogenetic analysis of GL1 indicates the presence of two diverged clades among the 26 accessions. Using ANOVA we show that the phenotypic variation of trichome density can not be explained by the sequence divergence between the two phylogenetic lineages. Sequence analysis of wildtype *A. thaliana* and *A. lyrata* accessions indicates that all 13 amino acid substitutions are located outside the conserved helix-turn-helix domains R2 and R3. Using plants of the two species *A. thaliana* and *A. lyrata* with either naturally occurring or EMS induced glabrous phenotypes, we demonstrate that the last 14 C-terminal amino acids of the GL1 gene have no major impact on the initiation of trichomes.

85 Evolutionary dynamics of resistance to bacterial pathogens

Joy Bergelson

University of Chicago

Plants and their pathogens are generally thought to participate in a coevolutionary arms race in which resistance alleles undergo a dynamical turnover in response to evolutionary changes on the part of pathogens. Under this evolutionary scenario, one would expect to see that alleles at an R-gene locus are young, and show evidence of strong directional selection driving their turnover. We have begun studying a set of R gene loci, including Rpm1, Rps2 and Rps5, to ask whether these predictions are borne out in population genetic samples. In particular, we have sequenced coding and flanking regions for each of these loci in approximately two dozen *A. thaliana* ecotypes representing a world-wide sample, as well as the outgroup *Arabis lyrata*. Surprisingly, we find that polymorphisms in resistance and susceptibility are maintained at these loci for millions of years and that there is no evidence of strong directional selection. These results suggest that balancing selection is acting to maintain both resistance and susceptibility. Potential mechanisms maintaining long-lived polymorphisms at R gene loci will be discussed.

86 A Modern Mustard Pack for Fighting Malaria

Jeffrey L. Blanchard

National Center for Genome Resources

The importance of *Arabidopsis* as a model system is extending beyond plants to the Apicomplexans, a group of parasitic protists that include the malarial parasites, coccidia,

piroplasms and Toxoplasma. The relevance of Arabidopsis research is not in the discovery of new plant medicinals, but in understanding basic cell biology. This surprising relationship is derived from the recent discovery of a non-photosynthetic plastid in all sampled members of the Apicomplexans. The plastid in Apicomplexans is the result of an endosymbiotic relationship between two eukaryotic microbes. In Apicomplexans the secondary endosymbiotic nuclear genome has completely disappeared over time, in part, through the transfer of the endosymbiont's nuclear genes to the host nucleus. Since the apicomplexan plastid is derived from secondary endosymbiosis, plastid proteins may have at least four separate evolutionary origins (the host nuclear genome, the endosymbiont's nuclear genome, the mitochondrial genome and the original cyanobacterial-derived endosymbiont). We know very little about the function of the plastid in Apicomplexans, but the plastid is an ideal target for drug therapies since many compounds that inhibit plastid activities kill Plasmodium and Toxoplasma. Our group is taking a computational approach by reconstructing Arabidopsis plastid metabolic pathways and using the data from the concurrent Arabidopsis, Toxoplasma and Plasmodium genome projects to discover common nuclear-encoded plastid-targeted proteins. We have already uncovered genes from all four evolutionary classes, as well as genes that seem to be "plant" like because of their origin from the endosymbiotic nuclear genome, but are not part of the plastid. This data is being integrated into PathDB (<http://www.ncgr.org/software/pathdb/>) a database of biochemical pathways and metabolism and we are developing and applying a software discovery tool to reveal new Arabidopsis and Apicomplexan metabolic pathways.

87 NASC

Keith Bradnam, Penny Dennis, Clair Hall, Jamie Kincaid, Stephanie Kitchingman, Karen Searle, and Sean May

NASC, BioSciences, University Park. Nottingham, NG7 2RD, UK

The Nottingham Arabidopsis Stock Centre [nasc.nott.ac.uk], in collaboration with the ABRC, act as safe repositories of research materials (primarily germplasm and DNA) for the world Arabidopsis community. We jointly seek out, amplify, curate and warehouse these donated resources, and then independently distribute them to Europe and America respectively and by collaboration to the rest of the world.

Between us, we currently hold insertion mutation stocks approaching transcriptome saturation as well as a wealth of characterised mutant and multiple mutant lines of Arabidopsis.

We have a wide variety of enhancer and promoter-trap marker lines (both GUS and GFP) available for expression studies and as markers of gene function, and have recently obtained a large number of sequence characterised insertion lines such as the SLAT and IMA populations whereby it is now possible to 'shop on-line' for your desired mutant genotype simply by text-matching a chosen gene or BAC sequence.

In addition, and of particular interest in this post-genomics period, we have hundreds of Arabidopsis ecotypes constituting a great deal of potential genetic variation. Tools available through us for phenotype/genotype analysis include several well-characterised mapping populations (Col/Ler; Cvi/Ler; Ws/W100F; and Col/Nd) which may be suitable for QTL investigation into your chosen visible or cryptic phenotype characteristics.

Appropriate marker scores are available at our web-site.

Our e-commerce 'shopping cart' system for ordering stocks has recently been upgraded to integrate smoothly with the Arabidopsis Genome Resource (AGR) - a part of [ukcrop.net]. You can now simply browse genome and insert information for germplasm stocks without having to learn complex software.

Our service will develop dramatically with the amalgamation of our catalogue with whole genome analysis and the array bioinformatics at NASC (part of UK functional genomics: GARnet [www.york.ac.uk/res/garnet/garnet.htm])

88 Epigenetic instability of Arabidopsis allopolyploid hybrids

Luca Comai, Steve H. Reynolds, Jerry Davison, and Ricardo Masuelli

Department of Botany, Box 355325, University of Washington, Seattle, WA 98195

Allopolyploid hybridization serves as a major pathway for plant evolution, but is associated in its early stages with phenotypic and genomic instabilities that are poorly understood. We have investigated the initial stages of allopolyploidization by characterizing synthetic allotetraploid hybrids between *Arabidopsis thaliana* ($n=2x=10$) and *Cardaminopsis arenosa* ($n=2x=16$). AFLP-cDNA analysis demonstrated gene silencing in the synthetic hybrids (20 silenced/700 genes examined). Three silenced genes were characterized by cloning the flanking regions. One, called K7, is similar to transposons and exists in multiple copies in each parental genome. Another matches RAP2.1, a gene that has numerous paralogs as well as a repeated element to its 5'. The last, L6, is an unknown gene close to ADH on chromosome I. CNG DNA methylation of K7 was lower in the hybrids than in the parents and the element varied in copy number. Reactivation of transcription in the hybrid progeny, indicated that the K7 elements are probably subject to epigenetic regulation. L6 was methylated in the *C. arenosa* genome. Analyses of DNA methylation and retroelements transcription suggest that anomalies in DNA methylation cause the reactivation of retrotransposons in the hybrids. The implications of transposons mobilization (hybrid dysgenesis) and gene silencing to allopolyploidization will be discussed.

89 DNA microarrays for expression profiling of osmotic stress responses

Michael K. Deyholos¹, Christine B. Michalowski², Gontran J. Zepeda Jr.², Chris Borchert², Susan Brazille², Hans J. Bohnert², David W. Galbraith¹

¹Dept. of Plant Science, and ²Dept. of Biochemistry, University of Arizona, Tucson AZ 85721

Drought and soil-salinity limit agricultural productivity in many parts of the world. To better understand the biological responses of osmotic stress, we are using microarrays to compare genomic expression patterns in several glycophytic and halophytic species. Here we describe the production and analysis of separate microarrays for the salt-sensitive species, *Arabidopsis thaliana*, and for the salt-tolerant species, *Mesembryanthemum crystallinum* (ice plant). Our *Arabidopsis* microarray contains over 14,000 elements, including clones from our own NaCl-treated EST libraries, and from the Arabidopsis Biological Research Center's minimized collection of ESTs 1. The ice plant microarray is comprised of 4,000 ESTs. We used these arrays to analyze changes in expression patterns at multiple time points, following NaCl treatment of each species. We also compared the effect of NaCl treatment in different tissues and at different developmental stages.

(1) Newman, T. et al., (1994) Genes Galore: A summary of methods for accessing results from large-scale partial sequencing of anonymous Arabidopsis cDNA clones. *Plant Physiology* 106: 1241-1255

90 The TIGR Gene Indices: Reconstruction and Annotation of Transcribed Sequences

Ingeborg Holt, Feng Liang, Geo Pertea, Svetlana Karamycheva, and John Quackenbus
The Institute for Genomic Research

A goal of the Genome Project is identification of the complete set of genes within each organism and the role played by these genes in development and disease. The sequencing of Expressed Sequence Tags (ESTs) has provided a first glimpse of the collection of transcribed sequences in a variety of organisms, but significant additional information can be obtained by a thorough analysis of the EST data. TIGR's analysis of the world's collection of EST sequence data, captured in the TIGR Gene Indices, provides assembled consensus sequences that are of high confidence and represent our best estimate of the collection of transcribed sequences underlying the ESTs. We maintain Gene Indices for a variety of species, including human, mouse, rat, *Drosophila*, zebrafish, rice, tomato, maize, soybean, and Arabidopsis. Collectively, the Gene Indices represent a unique resource for the comparative analysis of eukaryotic genes and may provide insight into gene function, regulation, and evolution.

Using the Tentative Consensus (TC) sequences in the Gene Indices, we recently developed the TIGR Orthologous Gene Alignment (TOGA) database. TOGA is designed to identify orthologous genes, including a significant number of novel orthologs, and to serve as a "cross-reference" between genomes, linking the Gene Indices for the surveyed species. This database represents the most extensive catalog of eukaryotic orthologues available, providing a valuable resource for gene identification, elucidation of functional domains, and analysis of gene and genome evolution.

91 Conserved C-Terminal Motifs of AP3 and PI are Important for Organ Identity Function

Rebecca S. Lamb and Vivian F. Irish

Department of Molecular, Cellular and Developmental Biology, Yale University, PO Box 208104, Osborn Memorial Laboratories, New Haven, CT 06520-8104

The APETALA3 (AP3) and PISTILLATA (PI) genes of Arabidopsis are necessary for petal and stamen development in the flower. They encode members of the plant MADS box family of putative transcription factors. AP3 and PI act as obligate heterodimers and presumably exert their organ identity function through transcriptional regulation of downstream genes. The plant MADS box proteins are characterized by the MADS DNA binding domain, an intervening region (the I box), an additional region of homology, the K box, and divergent C-termini. Despite the relatively high identity between the MADS box proteins involved in floral development and their similar *in vitro* DNA binding preferences, these proteins must regulate different downstream targets to make the different floral organs. One possible mechanism for generating specificity among these proteins is by binding different cofactors, which would help to refine their target specificity. Since the C-termini of these proteins are the most divergent in sequence, these regions are possible targets for cofactor binding. Previous work has suggested that the C-terminus

of AP3 may be necessary for full function of this protein and that the C-terminus of PI may also be important. Comparison of AP3 and PI orthologues from a wide range of species has identified short highly conserved motifs within the C-termini of both AP3 and PI. A series of truncation and domain swap constructs have been generated and expressed in the flowers of Arabidopsis to assess the importance of these motifs of AP3 and PI. Preliminary results suggest that these motifs are necessary for the full function of these proteins.

92 Mapping some economically important genes of oilseed rape with the aid of EST clones of Arabidopsis

Meng J, Zhao J, Xu F, Wang J, Wang L, Chen A, Zhou, Y

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University

Rapeseed is the most important oil crop with a very close relationship to Arabidopsis. Enormous information and precious resources developed from genome explore of model plant Arabidopsis would be superior valuable to genetic improvement of Brassica oil crops. One from the treasure of Arabidopsis is the expressed sequence tags, ESTs. We have been using some EST clones of Arabidopsis as probes along with other markers in Brassica to map some genes which control important characteristics in rapeseed.

Boron, one of the trace mineral elements, is essential to plant development and fertilization. The ability of boron using efficient is varied with cultivars and one major gene BE1 was found to be response to the character. From an F2 segregating population derived from Bankow X Qingyou 10, the gene BE1 is mapped into the linkage group LG9 in which 13 RFLP markers were detected with EST clones of Arabidopsis. The distance between the BE1 locus and the nearest EST locus is 0.8 cM. The homologous region of BE1 in Arabidopsis may probably be located on chromosome 1 by searching dbEST. We have sequenced the closely linked ESTs and the Brassica genomic clones. Sequence alignment showed that one of the genomic clone had 94% identity to Brassica juncea cDNA clone. Meanwhile, the pol CMS restore gene, Rf, was mapped into linkage group LG10 along with two Arabidopsis EST clones from a BC1 population of (1141A X 5200) X1141B. We also detected at least one QTL for the Sclerotinia sclerotium-resistance characteristic of B. napus although no EST clones of Arabidopsis has been found to be linked to the locus yet. Fine mapping of the three genes with more Arabidopsis EST probes is being currently carried out in the lab. .

93 Molecular evolution of the Arabidopsis and Brassica genomes

Carmel O'Neill, Anne-Marie van Dodeweerd and Ian Bancroft

Department of Brassica and Oilseeds Research, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH U.K.

The genome sequence of Arabidopsis thaliana (Arabidopsis) allows the analysis of the structure of an entire plant genome in unprecedented detail. As well as the expected high gene density, the genome sequence data have revealed an unexpectedly high level of genetic redundancy. In addition to tandem duplication of genes, larger-scale repetitive arrays of genes have been found and suggest that the "simple diploid" Arabidopsis may, in fact, be a degenerate tetraploid.

We have analysed a segmental duplication that was identified in the nucleotide sequences of *Arabidopsis* chromosomes 4 and 5. Although only 69% of genes are conserved between the segments, the order of those genes is perfectly conserved. We have also analysed, by detailed physical mapping, the corresponding segments of the genome of *Brassica oleracea* var *alboglabra*. Our studies confirm the hypothesis that the genomes of present-day *Brassica* species are derived from a triplication of an *Arabidopsis*-like genome. However, there is evidence for significant divergence of gene content. Our investigations indicate that the segmental duplication we have studied in *Arabidopsis* predates the divergence of *Arabidopsis* and the Brassicas, which occurred 10 - 35 million years ago. We have sequenced segments of four sets of paralogous/orthologous genes and found the expected high levels of sequence conservation and relationships between the individual genes. Further details of our results will be presented, along with the predictions we are now able to make about the extent of conservation that we are likely to find between the genomes of *Arabidopsis* and crop species.

94 Comparative genome analysis in crucifers

M. Rossberg, A. Acarkan, K. Boivin and R. Schmidt

Max-Delbrueck-Laboratory in the MPG, Carl-von-Linne-Weg 10, 50829 Cologne, Germany

Genome colinearity has been studied for two closely related diploid species of the Brassicaceae family, *Arabidopsis thaliana* and *Capsella rubella*. Comparative genetic mapping experiments revealed extensive colinear segments for the two species. Detailed analysis of a 200 kbp region in *A. thaliana* and its counterpart in *C. rubella* showed virtually complete conservation of gene repertoire, order, spacing and orientation. The comparison of orthologous genes revealed very similar exon-intron structures. Sequence identities of 90% or more were confined to exon sequences.

In the paleoploid species *Brassica oleracea* at least three partial copies were found corresponding to the 200 kbp *A. thaliana* region. Gene order in *B. oleracea* is similar to the one in *A. thaliana* and *C. rubella* for some genes, however, evidence for rearrangements was obtained. A characterisation of intergenic regions in *B. oleracea* showed a large variation in size when compared to the sizes in *A. thaliana* and *C. rubella*. Interestingly, for a number of genes only partial copies were found in *B. oleracea*. Virtually complete colinearity at the level of genes was found for the close relatives *A. thaliana* and *C. rubella*, whereas for the gene arrangement in the paleoploid species *B. oleracea* compared to the other two species differences were seen. Given the high degree of genome colinearity at the genetic level as well as at the molecular level in the Brassicaceae family, comparative genome mapping experiments can serve as an efficient means to transfer information and resources from the well-studied *A. thaliana* genome to related plants.

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

Randy Scholl, Doreen Ware, Emma Knee, Luz Rivero, Deborah Crist, and Staci Putney
Dept. of Plant Biology, The Ohio State University, Columbus, OH

The *Arabidopsis* Biological Resource Center (ABRC) maintains many stocks relevant to genome exploration. 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. A collection of 6500 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 50,000+, including enhancer trap lines from T. Jack, activation tagging lines from D. Weigel, enhancer trap lines from INRA (France), insertion lines from J. Alonso, W. Crosby and J. Ecker and lines from C. Koncz. The collection now includes major populations from the following donors: D. Weigel (22,000 lines), K. Feldmann (10,000 lines), T. Jack (11,300 lines) J. Ecker and J. Alonso (30,000 lines), C. Somerville and W. Scheible (50,000+ lines), M. Sussman and M. Amasino (60,624 lines) and R. Bressan (60,000 lines). Additional donations are expected in the next two years. DNA of 6,000 Feldmann lines has been distributed for three years, and DNA from 6,000 Jack lines has been available for two years. DNA for PCR screening will be extracted from additional lines. 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 users' results with PCR experiments will be presented. Progress in current DNA isolation and distribution efforts will be outlined. ABRC is supported by a grant from the National Science Foundation.

96 Comparative genetics of flowering time between *Arabidopsis* and *Brassica*: a tale of polyploidy, gene duplication and differential gene expression

M. Eric Schranz and Thomas C. Osborn

Dept. of Agronomy, University of Wisconsin-Madison

Arabidopsis thaliana and crop species in the genus *Brassica* are closely related, however, the brassicas have larger and more redundant genomes. The diploid brassicas such as *B. rapa* ($n=10$) and *B. oleracea* ($n=9$) are believed to be ancient polyploids. In addition, interspecific hybrids between diploid brassicas have given rise to higher polyploidy levels. For example, hybrids of *B. rapa* and *B. oleracea* yield the allopolyploid *B. napus* ($n=19$). Hence, genes that exist as single copy loci in *A. thaliana* can exist in 2, 4 or even more copies in an allopolyploid species such as *B. napus*. Higher levels of genetic redundancy may allow for greater adaptive plasticity and more flexibility for subsequent evolution. We have observed rapid evolution for variation in flowering time in plants descended from a single resynthesized *B. napus* polyploid after a few generations of self-pollination. To understand the genetic basis of this variation we have cloned homologs of key regulators of flowering time identified in *A. thaliana* (*FLC*, *CO*, *FCA*, *LFY*) from the parental diploids *B. rapa* and *B. oleracea* and from polyploid *B. napus*. Both *FLC* and *LFY* are duplicated in the diploid brassicas and there has not been significant sequence divergence between orthologs of the diploid and the polyploid loci. RT-PCR analyses of *FLC* reveals that the paralogs are both expressed in *B. rapa*, whereas only one locus is expressed in *B. oleracea*. Resynthesized *B. napus* polyploids show differential expression of paralogous *FLC* loci between early and late flowering selections.

97 Reproductive isolations between *Arabidopsis thaliana*, *Arabis*, and related species caused by defects in pollen tube guidance and embryogenesis

Kentaro K. Shimizu and Kiyotaka Okada

Department of Botany, Graduate School of Science, Kyoto University

Reproductive isolations (interspecific incompatibility) play critical roles in generating and maintaining species. It is thought to be caused by the divergence of any of the recognition molecules between female and male. Molecular genetic analyses of closely related species will reveal the mechanism of isolation, but it has been applied only to *Drosophila* species group. There remain several important questions. How many genes should change? What genes diverged? To address these questions, we collected 15 relatives of *A. thaliana* and analyzed the reproductive isolations as a model system. We examined the stages in which foreign pollen is rejected when crossed to *A. thaliana* pistils. (1) When a closely related species *Arabidopsis halleri* subsp. *gemma* was crossed, the embryogenesis was much delayed, suggesting a defect in the activation of paternal genes in the early embryogenesis. (2) *A. griffithiana* lies just outside the clade including *A. thaliana* and *A. gemma*. The efficiency of the pollen tube (PT) guidance was low, and the embryogenesis was aberrant as (1). (3) The PTs of *Cardamine* were occasionally entered the micropyle but did not fertilize. (4) The PTs of *Orychophragmus* wandered about. (5) The PTs of *Draba*, a distantly related species, had defect in the elongation in the transmitting tissue. In brief, each stage of fertilization process can be a critical step of the reproductive isolation. We overlaid the pattern on the phylogenetic tree and found a rule that the older the species divergence, the earlier stage the foreign pollen was rejected. This suggests that the mutations accumulated randomly, and thus supports a classic model that the speciation is a consequence of genetic divergence in reproduction genes between geographically isolated populations. Those results will be the basis of isolating the genes of reproductive isolation. As one approach, we isolated a series of mutants with defects in PTs in the pistil (see the abstract by K.Kanaoka, K.K.S. and K.O.). In *karakusa*, fertilization failed although PTs entered the micropyle as in the *Cardamine* cross. In *maa* mutants, PTs wandered as in the *Orychophragmus* cross. The mutated genes are candidates of key genes in the reproductive isolation.

98 Using global gene expression profiling to characterize the differences in *Arabidopsis* accessions

Vernadette A. Simon, Pam J. Green and Ellen Wisman

MSU-DOE Plant Research Laboratories, Michigan State University

Landsberg erecta and *Columbia* are commonly used *Arabidopsis* accessions.

Comparisons of the *Ler* and *Col* genomic sequences have demonstrated that there are many polymorphisms between them and because of this they have been used to construct high-density genetic maps for cloning. The developmental differences in *Landsberg* and *Columbia* have been studied extensively and a number of differently regulated developmental pathways have been described. To obtain a more global picture of these differences, we have assembled a microarray of 11,000 non-redundant EST sequences. We have isolated and labeled RNA from *Landsberg*, *Columbia* and hybrids of the two, and then hybridized that RNA onto our array. All plants were grown under similar conditions and, because of the differences in flowering time between the two accessions, the parental plants were harvested at the same developmental stage. We studied the variation between *Ler* and *Col* to determine what the differences in gene expression patterns are. In addition, we investigated the dominance effect in F1 plants generated from crossing *Ler* with *Col*. Here, we present the preliminary data of these experiments.

This project is supported by the NSF.

99 GARNet, Functional genomics in the UK

Karin van de Sande and Ottoline Leyser

University of York, Heslington, York YO10 5DD, United Kingdom

GARNet is a UK based Arabidopsis functional genomics network created to make full use of the information derived from the Arabidopsis Genome Initiative. As part of the BBSRC IGF (Investigating Gene Function) initiative, GARNet will be a national facility providing tools and services for functional Genomics. GARNet received BBSRC funding for a three year period, after which the services will continue on a cost recovery basis.

Transcriptome analysis, bioinformatics, metabolite profiling, proteome analysis and forward and reverse genetics are the spearpoints of the GARNet project. Further information on GARNet can be found at the GARNet website at

<http://www.york.ac.uk/res/garnet/garnet.htm>. On October the 2nd and 3rd, at the University of York the first of three GARNet annual users meeting will take place. The service providers will outline the service they provide, and external speakers will discuss the results they achieved using tools of functional genomics. A registration form is available on the website.

100 Identification of genes involved in desiccation tolerance and stress protection in *Arabidopsis thaliana*

Anita Berg and Reidunn B. Aalen

University of Oslo, Department of Biology, Division of Molecular Biology, P.O. Box 1031, Blindern N-0315 Oslo, Norway

Plants suffer from dehydration or water stress due to drought, low temperature or high salinity. To survive these environmental stress conditions, various biochemical and physiological responses are induced. A number of genes that respond to desiccation have been described, but to understand in more detail how cells achieve these adaptive responses, it is necessary to identify more genes involved in the osmosensory signal transduction pathways. Seeds acquire desiccation tolerance late in embryogenesis as an adaptive strategy to enable seed survival during storage or environmental stress, and to ensure better dissemination of the species. Seeds are thus a superb resource for the isolation and study of stress-related and stress-induced genes. There will be a demand for such genes for breeding purposes, if their orthologues can be isolated in economically interesting crop plants or transferred to them by gene technology. Using *Arabidopsis thaliana* as a model system, screening of a collection of T-DNA transformed lines has been initiated. Screening for mutants with decreased or increased sensitivity to osmotic and/or oxidative stress, has so far resulted in a number of candidate lines, which will be further analyzed. To identify the putative T-DNA tagged mutated genes, cloning of sequences flanking the T-DNA inserts is in progress.

101 Ozone: An abiotic elicitor of the hypersensitive response

Keith R. Davis and Mulpuri V. Rao

Paradigm Genetics, Inc., Bldg. 2, 104 Alexander Drive, RTP, NC 27709

Recent studies suggest that cross-talk between salicylic acid (SA)-, jasmonic acid (JA)- and ethylene-dependent signaling pathways regulate plant responses to both abiotic and biotic stress factors. Earlier studies demonstrated that the Arabidopsis ecotype, Cvi-0, exposed to O₃ exhibits an oxidative burst, hyperaccumulates SA, and develops lesions via activation of a hypersensitive (HR) cell death pathway. We have now confirmed the importance of SA in O₃-induced cell death and characterized the role of JA in influencing this response. Expression of salicylate hydroxylase (NahG) in Cvi-0 greatly reduced O₃-induced cell death. Methyl jasmonate (Me-JA) pre-treatment of Cvi-0 reduced O₃-induced H₂O₂ levels, SA content, PR1 mRNA accumulation, and completely abolished O₃-induced cell death. Cvi-0 synthesized as much JA as Col-0 in response to O₃ exposure, but exhibited greatly reduced sensitivity to exogenous Me-JA. Analyses of the responses of the JA-response mutants, jar1 and fad3/7/8, to O₃ showed that disruption of JA signaling in an O₃-tolerant Arabidopsis ecotype (Col-0) increases the magnitude of an O₃-induced oxidative burst, SA accumulation, and HR-like cell death. These results demonstrate an antagonistic relationship between JA and SA signaling pathways in controlling the magnitude of O₃-induced hypersensitive cell death.

102 Genetic dissection of phosphate sensing

Carla A. Delatorre, Carla A. Ticconi, and Steffen Abel

University of California, Davis

Phosphorus is one of the most important, yet least available, mineral nutrients required by plants. The element is an essential structural component of many biomolecules and functions at the nexus of photosynthesis, energy conservation and carbon metabolism. Plants have evolved elaborate metabolic and developmental adaptations to low phosphorus availability. Biochemical responses to phosphate (Pi) limitation include increased production and secretion of Pi acquisition proteins such as nucleases, acid phosphatases, and high-affinity Pi transporters. However, the signal transduction pathways that sense Pi availability and integrate the Pi starvation response (PSR) in plants are unknown. To genetically dissect Pi sensing, we have devised a conditional genetic screen for PSR-insensitive mutants and a selection scheme for PSR-constitutive mutants in Arabidopsis. Our screen is based on the facultative ability of wild type plants to metabolize exogenous nucleic acids when inorganic phosphate is limiting. After screening 50,000 M₂ seedlings, we have isolated twenty-two confirmed mutant lines that show severely impaired growth on medium containing DNA as the only source of phosphorus, but which recover on medium containing soluble Pi. Characterization of nine such mutant lines demonstrates inability to utilize either DNA or RNA. One mutant line, psr1, has significantly reduced activities of Pi starvation-inducible isoforms of ribonuclease and acid phosphatase under Pi limiting conditions. The data suggest that a subset of the selected mutations impair the expression of more than one Pi starvation-inducible enzyme required for utilization of exogenous nucleic acids, and may thus affect regulatory components of a Pi starvation response pathway in higher plants. The selection scheme for PSR-constitutive mutations takes advantage of phosphite, which is metabolically toxic but selectively represses Pi-starvation-inducible enzymes, including nucleases. Plants that survive on phosphite- and nucleic acid-containing medium are proposed to include mutations that cause constitutive expression and secretion of Pi-

starvation-inducible enzymes. Both classes of mutations will contribute to elucidating how plants sense phosphate availability.

103 The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3

Ursula Halfter, Manabu Ishitani, Jiping Liu and Jian-Kang Zhu

Dept. of Plant Sciences, University of Arizona, Tucson, AZ 85721

In *Arabidopsis thaliana*, the Salt Overly Sensitive 2 (SOS2) and Salt Overly Sensitive 3 (SOS3) genes are required for intracellular Na⁺ and K⁺ homeostasis. Mutations in these genes cause Na⁺ and K⁺ imbalance and render plants more sensitive toward growth inhibition by high Na⁺ and low K⁺ environments. Double mutant analysis indicates that SOS2 and SOS3 function in the same pathway. SOS3 is an EF-hand type calcium-binding protein having sequence similarities with animal neuronal calcium sensors and the yeast calcineurin B. Using the yeast two-hybrid system, we identified a group of related protein kinases that interact with SOS3. We have also isolated the SOS2 gene through positional cloning. SOS2 encodes a serine/threonine protein kinase in the SNF1/AMPK family. Interestingly, SOS2 kinase is similar to the kinase sequences identified in the yeast two-hybrid screen. SOS2 interacts with SOS3 in the yeast two-hybrid system and in *in vitro* binding assays. The interaction is mediated by the C-terminal regulatory domain of SOS2. SOS3 activates SOS2 protein kinase activity in a Ca²⁺-dependent manner. A SOS3 mutant defective in Ca²⁺-binding, corresponding to the loss-of-function allele, *sos3-1*, was not capable of activating SOS2. Therefore, SOS3 and SOS2 define a novel regulatory pathway important for the control of intracellular ion homeostasis in plants.

104 Regulatory elements in the ABA-, drought- and cold responsive Rab18 promoter of *Arabidopsis*

Elina Helenius¹, Sirpa Nuotio¹, Tiina Vahala², Pekka Heino¹ & E. Tapio Palva¹

¹Department of Biosciences, Division of Genetics, P.O.Box 56, FIN-00014 University of Helsinki, Finland ²Department of Forest Genetics, Swedish University of Agricultural Sciences (SLU), Box 7027, S-750 07 Uppsala, Sweden

We have employed the Rab18 gene as a model in order to analyse ABA-mediated gene expression during the cold acclimation of *Arabidopsis thaliana*. Rab18 encodes a hydrophilic, glycine-rich protein of the dehydrin family, thought to protect cellular structures from dehydration during cold and drought stress. The Rab18 transcript can be detected after 1-3 hours of desiccation and/or after application of exogenous ABA. Regulation of Rab18 is known to be ABA-dependent, since drought- and cold-induced Rab18 mRNA accumulation is blocked in the ABA-biosynthesis mutant *aba-1*. However, exogenous application of ABA can induce Rab18 also in *aba-1*, but not in ABA-insensitive response mutant *abi1*. The promoter region of Rab18 contains conserved sequence elements identified in other stress-inducible promoters. These elements include both the abscisic acid responsive element (ABRE) and the drought/low temperature responsive element (DRE/LTRE). In order to further identify important regulatory elements in the promoter, serial deletions of the 5' regulatory region of the Rab18 gene were fused upstream of the reporter gene GUS. Histochemical and fluorometric assays of the transgenic *Arabidopsis* plants suggest the presence of both positive and negative

regulatory elements in the promoter. The detailed analysis of the elements as well as isolation of corresponding transcriptional regulators binding these elements is in progress.

105 Multiple genes are required for the acquisition of thermotolerance in Arabidopsis
Suk-Whan Hong and Elizabeth Vierling
University of Arizona

Mechanisms to maintain metabolic homeostasis under fluctuating temperatures are likely to be of particular importance to plants, as plants are unable to move to more favorable environments. It has long been established that plants can adapt to otherwise lethal high temperatures if they are first subjected to pretreatments at more moderate, non-lethal high temperatures. This adaptation process is referred to as "induced" or "acquired" thermotolerance. We have begun a genetic dissection of acquired thermotolerance in Arabidopsis by isolation of loss-of-function mutants using a screen for adaptation of hypocotyl elongation after high temperature treatment. Four distinct loci, hot1,2,3 & 4, have been identified and confirmed to represent single recessive mutations by appropriate genetic and mapping studies. Back crossed, F3 progeny from each of the mutants were subjected to heat stress at different stages of growth and development to examine further the loss-of-thermotolerance phenotype. When seeds are heat stressed immediately after imbibition at 4°C, only hot1 seed fails to germinate, while hot2, 3 and 4 seed germinate like wild type. In tests of pretreated 10 day old plants, hot1 and hot2 cannot acquire thermotolerance, while hot3 and hot4 survive subsequent heat stress, remaining green like wild type. To study in vivo the recovery of a specific enzyme inactivated by heat, the gene encoding firefly luciferase (under control of the constitutive *ubc10* promoter) was introduced into wild type and hot mutant plants. After complete inactivation of luciferase by heat, plants were incubated for 1 to 4 hrs at 22°C and recovery of luciferase activity was monitored. hot1 and hot3 fail to recover luciferase activity, while hot2 recovers activity similar to wild type. The hot1 gene was identified by a map-based cloning and "candidate gene" strategy. hot1 encodes AtHsp101, a member of the Hsp100/ClpB family of proteins, which are required for thermotolerance in bacteria and yeast. The phenotype of hot1 provides direct evidence that AtHsp101 is also essential for thermotolerance in higher plants. In addition, the identification of other hot loci indicates that multiple processes, mediated by different genes are required for the development of thermotolerance at different life stages.

106 Overexpression of AtGluR2, an Arabidopsis homolog of mammalian ionotropic glutamate receptor, renders transgenic plants inefficient in nutrient utilization and hypersensitive to ionic stresses

Sun A Kim¹, Myeong-Hyeon Wang¹, Seul-Ki Jae¹, June M. Kwak^{1, 2}, Julian I. Schroeder² and Hong Gil Nam^{1*}

¹Division of Molecular Life Sciences, Pohang University of Science and Technology, Hyoja Dong, Pohang, Kyungbuk 790-784, Republic of Korea ²Department of Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA

In higher plants, ion homeostasis is important in the plant nutrition and the signal transduction, but a basic understanding of the molecular mechanisms for maintaining the ion homeostasis remains elusive. We have identified an Arabidopsis homolog, AtGluR2,

of mammalian ionotropic glutamate receptors and characterized the altered responses of transgenic plants in ion homeostasis-related physiology. The deduced polypeptide sequence, the hydropathy plot and the genomic structure of AtGluR2 demonstrate that the three-plus-one transmembrane structure and the modular structure are well conserved in AtGluR2 as in mammalian glutamate receptors. Transgenic Arabidopsis overexpressing AtGluR2 cDNA displayed symptoms of a specific ion starvation under composite soil growth conditions and hypersensitivity to a couple of other ions. Supplementation of the ion alleviated the ion deficiency symptoms caused by overexpression of AtGluR2 as well as hypersensitivity to ionic stresses, which indicates the inefficiency in the ion utilization in transgenic plants. The major macronutritional ions and metals in shoots were not different between control and transgenic plants, suggesting the ion uptake and root-to-shoot translocation are not affected in transgenic plants. The results suggest that AtGluR2 may play regulatory roles for ion distribution during the normal development and adaptation to ionic stresses.

107 Positional Cloning of A Negative Regulator of Cold Stress Signal Transduction
Hojoung Lee, Liming Xiong, Manabu Ishitani, Becky Stevenson, and Jian-Kang Zhu
University of Arizona

Low temperature is an important environmental factor affecting plant growth and development. Many genes are known to be induced by low temperatures. However, the signal transduction pathways leading to cold responsive gene transcription are poorly understood. We are interested in elucidating cold stress signal transduction pathways in plants. Previously, our group reported the characterization of an Arabidopsis mutant, *hos1-1*, which exhibits enhanced gene transcription under cold stress conditions. We have now isolated the HOS1 gene using a map-based cloning strategy. HOS1 encodes a protein with a RING-finger motif. Northern blot analysis showed that CBF1 genes are super-induced in *hos1-1* mutant plants. The results indicate that HOS1 is a negative regulator that functions upstream of CBF1 transcription factors in cold stress signaling pathways.

108 Mutations affecting hypoxic induction of fermentative and glycolytic genes
Ter-yun Lin, Hsiao-Ping Peng, and Ming-Che Shih
Department of Biological Sciences, 312 CB, University of Iowa, Iowa City, Iowa 52242
Expression of the alcohol dehydrogenase gene (ADH) of *Arabidopsis thaliana* is known to be induced by environmental stresses and regulated developmentally. We used a negative selection approach to isolate mutants that were defective in regulating the expression of the ADH gene during seed germination. A total of 18 mutants were obtained. Characterization of three recessive mutants, *aar1-1*, *aar1-2*, and *aar2-1* that belonged to two complementation groups indicated that mutations in AAR1 and AAR2 genes also affected anoxic and hypoxic induction of ADH. The *aar1* and *aar2* mutants were also defective in responding to cold and osmotic stress. Interestingly, the two allelic mutants, *aar1-1* and *aar1-2*, exhibited different phenotypes under cold and osmotic stresses. Based on our results we propose that these mutants are defective in a late step of the signaling pathways that lead to increased expression of the ADH gene. We have also examined the effects of the *aar* mutations on the expression of several glycolytic genes, including aldolase (ALD), triosephosphate isomerase (TPI), glyceraldehyde-3-phosphate

dehydrogeanse (GAPDH), and phosphoglycerate kinase (PGK). These gene products are required during both aerobic respiration and anaerobic fermentation, while ADH gene products are required only for alcoholic fermentation. Our data showed that mutations in some AAR genes affect hypoxic induction of both classes of genes. These results suggested that a common signaling pathway or at least shared signaling steps mediate the induction of these two different classes of genes in response to hypoxic stress.

109 Characterization of a cold-inducible gene encoding a putative monooxygenase in arabidopsis

R. M. Lopez-Cobollo, R. Catala, E. Santos, J. M. Martinez-Zapater and J. Salinas
Departamento de Mejora Genetica y Biotecnologia, Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA); Carretera de la Coruna, Km.7; 28040 Madrid; Spain

Arabidopsis is able to increase its tolerance to freezing in response to low-nonfreezing temperatures. This process, known as cold acclimation, is very complex and involves different physiological and biochemical changes, most of them being controlled by low temperatures through changes in gene expression. To understand the molecular mechanisms that control this process, we have isolated several cDNAs corresponding to genes which expression is induced by low temperatures at low/medium levels. Here, we present the molecular characterization of one of these cDNAs we named Rare Cold Inducible (RCI) 5A. Sequence analysis revealed that RCI5A encodes a protein showing similarity to monooxygenases. The deduced amino acid sequence of RCI5A shows a typical GXGXXG domain characteristic of flavin-containing monooxygenases (FMO), which has been involved in the binding of the ADP moiety of FAD. Northern-blot experiments indicated that RCI5A is positively regulated by low temperatures as well as dehydration and Clna but not by exogenous ABA. In order to better characterize the expression pattern of RCI5A, Arabidopsis transgenic plants containing a transcriptional fusion between a 770 pb promoter fragment and the uidA reporter gene were obtained. Analysis of these plants revealed that GUS activity accumulates under stress conditions, being mainly detected in vascular tissues. This indicates that the promoter fragment is sufficient to confer cold, drought and Clna regulation, and suggests that the regulation is occurring at the transcriptional level. Based on these results, and on the fact that FMO proteins have been related with a number of steps in the metabolism of phenolic compounds, we will discuss how the product of RCI5A may participate in cold acclimation leading to increased freezing tolerance.

110 GENETIC DISSECTION OF THE PHOSPHATE STARVATION SIGNALING PATHWAY IN ARABIDOPSIS

Ana C. Martín¹, Vicente Rubio¹, Roberto Solano¹, Juan Carlos Del Pozo^{1,2}, Joaquin Iglesias¹, Alicia de la Peña², Antonio Leyva¹, and Javier Paz-Ares¹

¹Centro Nacional de Biotecnologia-CSIC. Campus de Cantoblanco, 28049 Madrid, Spain. ² Departamento de Genetica. Facultad de Biologia. Universidad Complutense de Madrid. Ciudad Universitaria s/n. 28040 Madrid, Spain

Plants have evolved strategies to adapt growth under phosphate limiting conditions. These adaptative mechanisms involve developmental/morphological and metabolic/biochemical changes. Thus, plants grown under Pi limitation conditions

increase the root-shoot ratio, root hair length and lateral root number, and many plant species establish symbiotic associations with mycorrhizal fungi. Likewise, Pi starvation also produces changes in respiration rate, secretion of protons and organic acids and stimulation of phosphate transport, RNase and phosphatase activities. The regulatory system controlling these responses is poorly characterized. We have isolated the AtIPS1 (Induced by Pi Starvation) gene from *Arabidopsis thaliana*, which, like other members of the TPSII/Mt4 family, only contains short non-conserved reading frames and is highly specifically responsive to Pi starvation. An AtIPS1:GUS reporter gene was prepared also displaying a specific Pi starvation responsiveness. In order to identify signal transduction mutants, M2 progeny from 50,000 EMS mutagenised AtIPS1:GUS transgenic *Arabidopsis* seeds were screened using a non destructive system. We have identified 15 mutants displaying altered expression of the reporter gene, 2 showing constitutive GUS activity (type I) and 13 showing reduced Pi starvation responsiveness (type II). *atpho3* from class I and *atpho9* from class II had the broader effect on the Pi starvation response as evaluated by northern analysis of the expression of several Pi starvation responsive genes. *atpho9* was shown to be epistatic over *atpho3*, suggesting that AtPHO9 acts downstream of (or at the same level than) AtPHO3. AtPHO3 and AtPHO9 have been mapped to chromosomes 4 and 3, respectively, providing a first step towards the cloning and molecular characterisation of components of the Pi starvation signal transduction pathway

111 Signal Transduction Components of the Unfolded Protein Response

Immaculada Martinez, Nozomu Koizumi and Maarten J. Chrispeels

Biology Department, Univ. of Calif. San Diego. La Jolla, CA 92093-0116. USA

The unfolded protein response (UPR) mediates signaling from the endoplasmic reticulum (ER) to the nucleus, resulting in a transcriptional induction of ER chaperones such as BiP. In yeast, this pathway is initiated by the transmembrane protein Ire1 that has a luminal sensor domain and a cytosolic kinase/endoribonuclease domain. From *Arabidopsis*, we isolated two homologous Ire1 cDNAs, AtIre1.1 and AtIre1.2, which have 70% identity in their cytosolic C-terminal portions. These AtIre genes also share 35% identity with the human, *C. elegans* and yeast proteins in their cytosolic domains. The luminal sensor domains of the proteins are less conserved. We made transgenic plants that express a BiP::GUS fusion and in these plants GUS is induced by tunicamycin, a glycosylation inhibitor that induces the UPR. This provides a convenient assay system to detect the UPR. To check the putative function of Ire1.1p in *Arabidopsis*, we introduced the AtIre1.1 gene driven by the 35S promoter in these BiP-GUS plants. No induction of the GUS expression was detected in the presence or absence of tunicamycin. There was also no upregulation of the mRNA level, which is extremely low in the wt. Expression of the Ire1 gene may be autoregulated, possibly by its own kinase domain, preventing any overexpression of the protein. Thus it may not be possible to create plants with a constitutive UPR. A yeast one-hybrid system was used to identify a transcription factor that binds to the UPR element present in the BiP promoter. The same factor was isolated three times independently. The function of this transcription factor in the UPR needs to be confirmed. Fusion proteins between the AtIre1.1 gene and GFP were generated to localize the protein and preliminary results confirm the ER localization of

AtIre1p. Finally, knockouts for both genes are being screened using the Arabidopsis insertion mutant collection of the University of Wisconsin.

112 Screening of Activation T-DNA Tagged Arabidopsis thaliana to Investigate the Determinants of Stress Responses

McCully MG*, Barb AW*, Koiwa H, Zhu J-H, Yokoi S, Reddy M, Rus A, Ruggeiro B, Sokolchik I, Sharkhuu A, Bressan RA, Hasegawa PM

Center for Plant Environmental Stress Physiology, Department of Horticulture and Landscape Architecture, Purdue University, 1165 Horticulture Building, West Lafayette, Indiana, 47907-1165

Insertion of activation T-DNA tags results in two possible significant genetic mutations: endogenous gene activation or disruption resulting in altered function. More than 130,000 T-DNA tagged lines of Arabidopsis thaliana ecotype C24, containing a stress responsive promoter-luciferase reporter fusion (RD29A::LUC), and more than 100,000 plants of the Columbia ecotype salt overly sensitive (sos3) mutant (both genotypes provided by J-K Zhu, University of Arizona), have been obtained by in planta floral dip transformation mediated by Agrobacterium carrying the activation tagging vector pSKI15 (provided by D. Weigel, Salk Institute). Our high throughput screening is focused principally on altered salt sensitivity/tolerance or regulated expression of the stress responsive RD29A promoter-reporter by CCD imaging. Salt sensitive mutants are identified using the root-bending assay, in which one week-old seedlings are transferred to salt containing medium (140 mM NaCl) and evaluated after one week. Salt tolerant mutants are identified by assessing the ability to germinate on salt medium (145 mM NaCl). Stress signal transduction mutants are identified by hyper-, hypo- or constitutive induction of RD29A::LUC expression by cold (0°C for two days) or ABA treatment (100 μ M) using a CCD imaging system. Identification of sos3 suppressors is the goal of the gain-of-function screening of the Columbia sos3 tagged populations. Physiological, molecular and genetic characterization of several mutants is in progress.

Supported by NSF Plant Genomics Initiative #DBI-98-1336, and JSPS Junior Scientist fellowship (SY).

*these authors contributed equally to this work.

113 The engineered phytoremediation of ionic and methylmercury pollution

R. B. Meagher*, S.P. Bizily, C.L. Rugh, A.C.P. Heaton, and N.J. Wang

Department of Genetics, University of Georgia, Athens, GA. 30602.

<http://www.genetics.uga.edu/RBMSite/rbmlabhome.html>

Plants have many advantages as agents for the remediation of environmental pollutants including: a rapidly growing root system, the natural ability to extract nutrients from the soil to above ground tissues, and the use of photosynthetic energy to power the process. Our long-term goal is to enable highly productive plant species to extract, resist, detoxify, and/or sequester toxic heavy metal pollutants. We have focused our research on the phytoremediation of soil and water-borne mercury. Mercury pollution is a serious world-wide problem affecting the health of human and wildlife populations. Using our experience with Arabidopsis as a model, several diverse plant species have been engineered to use a highly modified bacterial mercuric ion reductase gene, merA, to

detoxify ionic mercury (Hg(II)) by reducing it to Hg(0). These plants germinate, grow, and set seed at normal growth rates on levels of Hg(II) that are lethal to normal plants. Physiological experiments suggest these plants can be engineered to either transpire Hg(0) from leaves or trap Hg(II) in leaf tissues. However, a more serious problem than Hg(II) is the methylmercury (MeHg) produced by native bacteria in mercury-contaminated aquatic and marine wetlands. MeHg is more toxic than Hg(II), is efficiently biomagnified over several orders of magnitude in the food chain, and poses the most immediate threat to animal populations. In order to degrade this toxin, we have engineered three plant species, Arabidopsis, tobacco, and rice, with a modified bacterial organomercurial lyase gene (*merB*) to degrade methylmercury (MeHg) to the less toxic Hg(II). Many of the plants examined are highly resistant to MeHg and degrade it to Hg(II). An extensive array of molecular physiology experiments are underway to characterize the expression of *merA* and *merB* in transgenic plants. Our work suggests that native macrophytes (e.g., trees, shrubs, grasses) can be engineered to thrive on and detoxify the most abundant forms of ionic and organic mercury at polluted sites.

114 Herbicides mode of action in Arabidopsis

Ruth Meissner, Rita Froede, Christoph Bruhn, Anja Leihkauf, Gisela Lersch, Nicole Schumacher, Susanne Winkler, Christa Lechelt-Kunze
Bayer-AG, ZF-BTB, Gebaeude Q18, D-51368 Leverkusen

Herbicides comprise 50% of the agrochemical market. However, the amount of validated targets addressed by known herbicides is relatively low. Therefore the interest in new herbicidal compounds and targets is huge. As classical biochemical research only partly satisfies this demand, new approaches are necessary in the search of new herbicidal targets. These can involve genomics and bioinformatics as well as recent advances in analytical techniques.

In our approach the effects on gene expression and plant metabolism of herbicides with known and unknown mode of action are tested on *Arabidopsis thaliana*. To establish the project, glyphosate and chlorsulfuron two herbicides with known mode of action have been investigated. Glyphosate inhibits EPSPS (5-enoylpyruvylshikimic acid-3-phosphate synthase) an enzyme of the aromatic amino acid synthesis pathway, whereas chlorsulfuron inhibits the ALS (acetolactate synthase) an enzyme of the branched amino acid synthesis pathway. Plants were sprayed with the respective herbicide and harvested in a time course after treatment. Changes in gene expression of treated plants versus control plants were detected by identifying differentially expressed genes in subtractive cDNA libraries. Changes in metabolic composition were revealed by comparison of metabolic profiles of amino and organic acids. Analysing the resulting data, especially of the early harvesting timepoints, we were able to pinpoint the correct pathways responding to the chemicals applied. Later harvesting timepoints resulted in huge amounts of differentially expressed genes and an enormous increase of all amino acids reflecting the general physiology of dying plants rather than specific herbicidal effects.

115 Promoter analysis of the *erd1* gene encoding Clp protease regulatory subunit homolog under senescence and dehydration in Arabidopsis

Kazuo Nakashima¹, Yoshihiro Narusaka¹, Sean D. Simpsom¹, Kazuko Yamaguchi-Shinozaki¹ and Kazuo Shinozaki²

1Japan International Research Center for Agricultural Sciences (JIRCAS) and 2The Institute of Physical and Chemical Research (RIKEN)

The *erd1* gene, encoding a protein with sequence homology to the ATPase regulatory subunit of Clp protease, is strongly induced by dehydration and high salinity stress but not by heat, cold or heavy-metal stress in *Arabidopsis*. The *erd1* gene is also up-regulated during natural senescence and dark-induced senescence. To analyze the regulatory mechanisms involved in *erd1* gene expression by water stress and during senescence, we constructed a chimeric gene with the promoter region (-723 to +165) of the *erd1* gene fused to the LUC (luciferase) reporter gene and introduced them into tobacco and *Arabidopsis* plants. Expression analysis of the LUC gene in the transgenic plants incubated in dark conditions for 12-14 days revealed that the 69-bp region of the *erd1* promoter might contain cis-acting elements that are involved in senescence-promoting expression of the *erd1* gene. The 69-bp region contains the ABRE (ABA Responsive Element)-like sequence (ACGTG) and another ACGT sequence. The 69-bp region did not promote LUC gene expression in response to dehydration, high salinity stress or abscisic acid (ABA) treatments. Expression analysis of this region containing a base-substituted ABRE-like sequence or a base-substituted ACGT sequence indicated that both of these sequences might be involved in gene expression during dark-induced senescence in *Arabidopsis*. On the other hand, we are analyzing cis-acting elements involved in the dehydration-induced expression using deleted or base substituted DNA fragments of the *erd1* promoter fused LUC genes.

116 The relationship 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*

Narusaka, Y.1, Nakashima, K.1, Abe, H.1, Furihata, T.1, Sakuma, Y.1, Shinwari, K. Z.1, Yamaguchi-Shinozaki, K.1 and Shinozaki, K. 2

1JIRCAS, Tsukuba 305-8686, 2RIKEN, Tsukuba 305-0074

In order to understand the 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 ; TACCGACAT). DRE is not only involved in gene induction by dehydration and high salinity, but also by low temperature stress. However, DRE is not involved in ABA-responsive gene expression. The ABRE (ABA Responsive Element ; TACGTGTC) is thought to be one of cis-acting elements in ABA-dependent expression of *rd29A*. We analyzed the promoter region (-190 to -71) of *rd29A* containing the DRE, DRE-like (AGCCGACAC) and ABRE sequences, and examined the relationship between DRE and ABRE. Gel-shift assays revealed that DRE binding proteins (DREB) 1 and 2, not only bound to DRE, but also to the DRE-like sequence. The promoter region (-173 to -71) of *rd29A* containing the DRE-like sequence but not DRE responded to dehydration, high salt and low temperature stresses. These results suggest that the DRE-like sequence is also able to function as a DRE. In addition, we have isolated cDNAs encoding two ABRE binding proteins (AREB1 and 2). Co-expression experiments using *Arabidopsis* protoplasts showed that DREBs and AREBs cumulatively transactivated the expression

of a GUS reporter gene fused a region of the rd29A promoter containing DRE and ABRE. We discuss the relationship between the two cis-acting elements, DRE and ABRE.

117 Superoxide-driven cell death in the ozone-sensitive arabidopsis mutant rcd1 is ethylene-dependent and counteracted by jasmonate

Overmyer, K.1 Tuominen, H.1 Kettunen, R.1 Langebartels, C.2 & Kangasjärvi, J.1
1Institute of Biotechnology, University of Helsinki, POB 56 (Viikinkaari 5 D), FIN-00014 Helsinki, Finland; 2Institute of Biochemical Plant Pathology, GSF Research Center, D-85764 Oberschleissheim, Germany

The Arabidopsis mutant rcd1 (radical-induced cell death) was isolated on the basis of its ozone sensitivity. rcd1 shows hypersensitivity to extracellular superoxide but not to H₂O₂. Short term treatments of rcd1 with ozone, exogenous superoxide, or interaction with an incompatible bacterial pathogen cause massive foliar cell death. This cell death, that continues to spread transiently even after the triggering stimulus has ceased, is superoxide dependent and associated with a front of extracellular superoxide accumulating in advance of the lesioned tissue. Exogenous ethylene promotes the radical-induced cell death in both rcd1 and the Col-0 wild type, but not in the ethylene-insensitive ein2 mutant. Very high ozone results in massive damage and superoxide accumulation in Col-0 while ein2 remains tolerant. Furthermore, rcd1/ein2 double mutant, or ozone exposed rcd1, treated with the ethylene antagonist norbornadiene do not develop lesions. Thus, we have shown that superoxide accumulation and associated cell death are ethylene dependent. Differences in gene expression patterns between Col-0 and rcd1 suggested alterations in jasmonate (JA) -related processes. The JA-insensitive mutant jar1 was found to be radical sensitive, and, like rcd1, accumulated superoxide at lesion borders. Treatment of rcd1 with JA after ozone exposure resulted in the containment of lesion spread, further implying a protective role for JA. These results show that ethylene acts as a pro-death factor and regulates the superoxide dependent lesion development during the lesion propagation phase, while JA has a protective anti-death role. Interaction between these pathways may serve to fine-tune the relative contribution of propagation and containment processes in the regulation of radical-driven cell death and is a current topic under further investigation in our laboratory.

118 Ectopic expression of ABI3 gene enhances freezing tolerance in response to abscisic acid and low temperature in Arabidopsis thaliana

Ilkka Tamminen¹, Pirjo Mäkelä¹, Pekka Heino^{1,2} and E. Tapio Palva^{1,2}

¹ Department of Biosciences, Division of Genetics and ² Institute of Biotechnology, P.O. Box 56, FIN-00014 University of Helsinki, Finland

The plant hormone abscisic acid (ABA) regulates physiological and developmental processes in plants including stress adaptation and seed maturation. ABA-mediated processes appear central in plant cold acclimation and expression of cold acclimation related genes. Ectopic expression of ABI3 encoding a seed specific transcriptional activator confers the ability to Arabidopsis vegetative tissues to accumulate seed specific transcripts in response to ABA and also influenced some ABA-mediated vegetative responses. In the present study we characterized the effect of ectopic expression of ABI3 on cold acclimation and development of freezing tolerance in Arabidopsis. We first

characterized the effect of ABI3 on ABA-induced expression of cold acclimation related genes. Expression of ABI3 increased the ABA-induced accumulation of transcripts for several ABA/cold/drought responsive genes. Enhanced expression of such genes was evident even with transient application of ABA. This enhanced expression of genes was correlated with increased freezing tolerance in ABI3 transgenic plants. This enhanced development of tolerance was achieved even by transient application of ABA. Ectopic expression of ABI3 appeared also to modulate low temperature-induced freezing tolerance. The ABI3 transgenic plants acclimated faster than the wild-type plants and the maximum tolerance obtained was significantly higher. These data show that lower levels of ABA were needed to trigger expression of the genes and maintain the freezing tolerant state in the transgenic plants. This indicates that ectopic expression of ABI3 leads to enhanced responsiveness to ABA. This would also provide a new strategy for engineering stress tolerance.

119 Regulation of ozone sensitivity and ozone-induced gene expression by ethylene, jasmonic acid and salicylic acid in Arabidopsis mutants

Tuominen, H., Overmyer, K. and Kangasjärvi, J.

Institute of Biotechnology, University of Helsinki, POB 56 (Viikinkaari 5D), 00014 Helsinki, Finland

Ozone is an air pollutant, which induces oxyradical production and signaling processes inside the cells in much the same way as in the hypersensitive pathogen response. Signaling molecules that are known to be involved include ethylene, jasmonates and salicylic acid. These signaling pathways induce expression of various defense and stress-related genes. Ethylene production enhances ozone-induced lesion formation, and functional ethylene signaling is required in this process. In an opposite manner, jasmonates are believed to protect plants from ozone damage through containment of the ozone-induced lesions. Salicylic acid seems to have a dual role in damage formation since salicylates are involved in both stress protection and potentiation of oxidative burst and cell death. We have isolated four ozone-sensitive Arabidopsis mutant lines that display various degrees of lesion formation in response to a 250-ppb ozone treatment. Defense responses are not defective in these mutants as determined by superoxidase dismutase activity, ascorbate concentrations and expression of defense-related genes. A cDNA array of 92 genes revealed upregulation of some of the pathogenesis-related proteins in three of the mutant lines (ml2-40, ml2-11, ml2-3). Mutant line 2-40 had additional changes in salicylate-related gene expression, and rcd1 (for radical-induced cell death) and ml2-11 in jasmonate-related gene expression. Ozone sensitivity of known Arabidopsis signaling mutants was also tested. Compared to Columbia wild type, ethylene-insensitive *etr1-1* and *ein2* were more tolerant to ozone, jasmonate-resistant *jar1* more sensitive to ozone, and salicylate-deficient transgenic NahG line and salicylate-insensitive *npr1* mutant were similar to the wild type. A cDNA macroarray analysis of 144 genes revealed involvement and complex interaction between ethylene, methyl jasmonate and salicylic acid in the regulation of ozone-induced gene expression. These results will be discussed in relation to ethylene emission and the quantity of salicylates and jasmonates in these mutants.

120 Arabidopsis mutants deficient in mitochondria-nucleus communication

Jianping Yu, Roxy Nickels, Lori Viculis, and Lee McIntosh
MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI
48824, USA

More than eighty percent of the mitochondrial proteins are encoded by nuclear genes, synthesized in cytoplasm, and transported into mitochondria. Furthermore, Many mitochondrial-encoded proteins do not work alone. They instead form protein complexes with subunits encoded by nucleus, and function in respiration. Consequently, mitochondria must control the expression of nuclear genes according to the functional states of mitochondria. However, little is known about the communication pathways between mitochondria and nucleus in plants. An example for mitochondrial-regulated nuclear gene expression in plants is the gene *aox*, which encodes a mitochondrial protein the alternative oxidase (AOX). Expression of *aox* is upregulated under a variety of stress conditions, and in response to a mitochondrial specific inhibitor antimycin A. To initiate a genetic analysis of the communication between mitochondria and nucleus in plants, we have developed a screen for Arabidopsis mutants that are deficient in antimycin A induced AOX expression. We screened the T-DNA tagged lines generated by Thomas Jack's laboratory at Dartmouth College. Eleven putative mutants, with three phenotypes, have been identified. Seven of them have been successfully rescued. Six independent lines are impaired in antimycin A induced AOX protein accumulation, indicating a lesion in mitochondria-nucleus communication. Some mutants showed altered cotyledon and root morphology and partial sterility. The T-DNA insertion sites in these mutants are being characterized, and genetic analysis is in progress.

121 Structure-Function Analysis of the RPS5-Mediated Disease Resistance Protein in Arabidopsis

Jules Ade, Michal Swiderski, William Underwood and Roger Innes
Department of Biology, Indiana University, Bloomington, IN 47405-3700

In the gene-for-gene disease resistance model, it has been widely speculated that resistance gene (R gene) products function as receptors for protein ligands encoded by pathogen avirulence (*avr*) genes. However, this hypothesis has not yet been proven. We are working to provide insights into the mechanisms by which R genes activate defense responses in plants. Previous work has shown that the Arabidopsis RPS5 gene product, especially its third LRR, mediates resistance against the pathogen *Pseudomonas syringae* carrying the avirulence gene (*avrPphB*), and that it may interact with a signal component shared by multiple R gene pathways (Warren et al. (1998) *Plant Cell* 10: 1439-1452). Our goal is to determine the plant protein(s) that interact with RPS5 to trigger disease resistance. In one strategy, we are using in vitro coupled transcription/translation to produce RPS5 and putative interacting-proteins in vitro. We are currently examining whether these proteins can be co-immunoprecipitated with RPS5 using an RPS5 polyclonal antibody. In a second strategy, we are attempting to co-immunoprecipitate RPS5 with its interacting-proteins directly from plant tissue. Finally, we are using PCR-based mutagenesis of the third LRR of RPS5 in order to recover dominant negative and constitutive signaling mutations.

122 MUTATIONAL ANALYSIS OF TURNIP CRINKLE VIRUS MOVEMENT PROTEIN P8

Muslum Akgoz and Kristin K. Wobbe

Worcester Polytechnic Institute, Worcester, MA 01609

Turnip crinkle virus encodes two proteins, p8 and p9, that are both required for cell-to-cell movement. The p8 movement protein has been demonstrated to bind RNA in a cooperative manner, although, similar to many other plant virus movement proteins, it contains no canonical RNA binding domain(s). However, three positively charged regions of p8 potentially form ionic interactions with the RNA backbone. To identify functional regions of p8, a series of alanine and deletion scanning mutations were produced. The effects of these mutations were analyzed using both in vitro RNA binding assays and in vivo infections of susceptible (Di-3) and resistant (Di-17) *A. thaliana* plants. Several mutants that have reduced RNA binding ability were also demonstrated to be movement deficient and replication competent. Based on these results, there appear to be two RNA binding regions, located between amino acids 18 and 31, and 50 and 72. Single amino acid changes of the non-infectious mutants have been created and they will be investigated to determine individual interactions of p8 with the movement machinery.

123 DNA microarray analysis reveals overlapping patterns of gene induction in *Arabidopsis thaliana* following fungal challenge and treatment with defense signaling molecules

Jonathan P. Anderson¹, Peer M. Schenk², Kemal Kazan², Iain Wilson³, Shauna C. Somerville³ and John M. Manners²

¹The University of Queensland, St Lucia, Qld 4072, Australia, ²CSIRO Tropical Agriculture, Brisbane, Australia ³Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA 94305, USA

Changes in the expression patterns of 2375 selected genes were examined simultaneously by cDNA microarray analysis in *Arabidopsis thaliana* cv. Columbia plants after inoculation with an incompatible fungal pathogen *Alternaria brassicicola* or treatment with the defense-related signalling molecules salicylic acid (SA), methyl jasmonate (MJ) or ethylene (Eth). Substantial changes (up- and down-regulation) in the steady state abundance of 705 mRNAs was observed. In leaf tissue inoculated with *A. brassicicola*, the abundance of 168 mRNAs was increased more than 2.5-fold while those of 39 mRNAs were reduced. Similarly, the abundance of 192, 221 and 55 mRNAs was highly (2.5-fold) increased following treatment with SA, MJ and ethylene, respectively. Microarray analysis confirmed the expression patterns of numerous genes known to have defensive functions. In addition, 106 genes with no previously described function or homology to other genes displayed differential expression in response to one or more of the treatments, suggesting their possible involvement in plant defense responses. Arrangement of the genes into co-induced or co-repressed clusters for each of the treatments revealed the expression of many genes were influenced by multiple treatments/defense pathways. The largest number of genes co-induced and co-repressed was observed for the SA and MJ treatments where 55 genes (approximately 15.4% of genes induced by either SA or MJ independently) were co-regulated. This was followed by 25 genes co-induced by MJ and Eth treatments (10 % of genes induced by the individual treatments) and 17 genes co-induced by SA and Eth treatments (7.4% of individually induced genes). Ten genes were induced by all the chemical signal treatments. These results indicated the existence of substantial regulatory interactions and

cross-talk occurring during plant defense among the different defense signalling pathways.

124 Cloning and Characterization of CPR5

Lisa K. Anderson, Scott A. Bowling, and Xinnian Dong

Dept. of Botany, Duke University

The *cpr5* mutant (constitutive expressor of PR genes) is characterized by constitutive resistance to *Pseudomonas syringae* pv. *maculicola* ES4326 and *Peronospora parasitica* Noco2, elevated levels of salicylic acid (SA), reduced trichomes and spontaneous formation of lesions that mimic the hypersensitive response. The CPR5 gene was cloned using a map-based approach. The full length genomic sequence complemented all *cpr5* mutant phenotypes. The full length cDNA was obtained and transformed into *cpr5* and Columbia WT plants. CPR5 encodes a 62kd protein with five potential transmembrane domains and has little similarity to known proteins. Expression and localization data will be discussed.

Boch, J., Verbsky, M.L., Robertson, T.L., Larkin, J.C., and Kunkel, B.N. (1998) Analysis of Resistance Gene Mediated Responses in *Arabidopsis thaliana* plants carrying a mutation in CPR5. *Molecular Plant Microbe Interactions*. 11: 1196-1206.

Bowling, S.A., Clarke, J.D., Liu, Y., Klessig, D.F., and Dong, X. (1997) The *cpr5* Mutant of *Arabidopsis* Expresses Both NPR1-Dependent and NPR1-Independent Resistance. *Plant Cell*. 9: 1-13.

125 A Loss of Function Allelic Series for the Bacterial Avirulence Gene *avrRpt2* and the *Arabidopsis* Disease Resistance Gene RPS2

Axtell Michael J1, Mudgett, Mary Beth1, McNellis, Timothy2, and Staskawicz Brian J1

1: Department of Plant and Microbial Biology, University of California, Berkeley, USA

2: Department of Plant Pathology, The Pennsylvania State University, USA

Plant disease resistance is often mediated by the gene-for-gene interaction of plant resistance genes and cognate bacterial avirulence genes. *Pseudomonas syringae* pv. *tomato* bacteria expressing the *avrRpt2* avirulence gene trigger a hypersensitive response (HR) in *Arabidopsis* plants that have the corresponding RPS2 disease resistance gene. Transgenic *Arabidopsis* engineered for inducible expression of *avrRpt2* display hypersensitive cell death dependent on the presence of RPS2 in the genetic background. We have screened over 300,000 mutagenized (M2) seed for *Arabidopsis* (ecotype Col-0) mutants that fail to respond to *avrRpt2* expression, and isolated over 100 individual mutant lines. Several of these lines retain the ability to recognize bacteria expressing *avrRpt2*; Eight of these lines have been shown to harbor mutations within the inducible *avrRpt2* transgene. All mutant lines that fail to recognize *avrRpt2* bearing bacteria also fail to complement *rps2-101C* plants, indicating allelism to RPS2. Ten out of ten such mutants analyzed contain a mutation in the RPS2 open reading frame. Three of the novel RPS2 mutants also fail to complement *ndr1-1* plants or the parental Col-0 wild-type, and are being investigated as possible dominant-negative alleles of RPS2. Thus, this screen has quickly revealed a loss-of-function allelic series for both *avrRpt2* and RPS2, and has allowed a detailed analysis of the structure-function relationship of the RPS2 gene product.

126 Identification and cloning of three avirulence genes from *Pseudomonas syringae* pv. tomato strain DC3000

Sruti Bandyopadhyay and Sheng Yang He

Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48823

Pseudomonas syringae pv. tomato DC3000 (Pst DC3000) is a pathogen that uses the type III secretion system to infect the model plant *Arabidopsis thaliana*. The type III secretion system is broadly conserved in bacterial pathogens of plants and animals and delivers bacterial proteins into the host cell. This system is used by Pst DC3000 to transport virulence and avirulence factors into the host cell. The avirulence (Avr) proteins are recognized by host disease resistance gene products, leading to the elicitation of HR and resistance in host plants. Interestingly, many avr genes have been shown to be required for bacterial virulence in susceptible hosts. However, of all the known avr genes, only two, avrPto and avrE, have been found in Pst DC3000. In fact, it is now believed that avr gene products are collectively responsible for type III secretion specific bacterial virulence in a susceptible host.

We screened a cosmid library of Pst DC3000 to discover additional avirulence/virulence genes of Pst DC3000 using all known avirulence genes. We have identified and cloned the homologues of two avirulence/virulence genes, avrPphE and virPphA from *Pseudomonas syringae* pv. phaseolicola, and avrPpiB from *Pseudomonas syringae* pv. pisi in Pst DC3000. Sequence data of Pst virPphA and Pst avrPphE reveal a conserved harp box in the putative promoter region which implies the requirement of a functional hrpL for gene regulation. We are currently generating Pst DC3000 mutants lacking these three genes. Characterization, in planta growth and hrp dependency of these mutants will be presented.

127 Allele-specific interaction between the LRR domain of RPS2 and other defense-altering loci, including a locus that may also contribute to RPM1 function

Diya Banerjee^{1,2}, Xiaochun Zhang³ and Andrew Bent^{1,2,3}

¹Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706. ²Physiological and Molecular Plant Biology Program, University of Illinois, Urbana, IL. ³Dept. of Crop Sciences, Univ. of Illinois, Urbana, IL, 61801

The resistant ecotype Col-0 and the susceptible ecotype Po-1 were used to identify additional *Arabidopsis* loci that function in the avrRpt2-RPS2 and avrRpm1-RPM1 resistance pathways. Segregation of multiple genes controlling resistance to *P. syringae* avrRpt2+ or *P.s.* avrRpm1+ was hypothesized when Po-1 x Col-0 F2 segregation ratios did not fit single-gene models and plants with intermediate resistance phenotypes were observed. Some F2 individuals/F3 families were homozygous for Po-1 RPS2 but resistant to *P.s.* avrRpt2+, or homozygous for Col-0 RPS2 and yet disease susceptible. These individuals indicate the involvement of genes other than RPS2 in the resistance response, and also indicate allele-specific interactions between RPS2 and these other loci. The functionality of the Po-1 allele of RPS2 in non-Po-1 genetic backgrounds was confirmed via transformation of Col-0 rps2/rps2 mutants. Col-0 RPS2 complemented Po-1 plants for disease resistance, suggesting that the deficient resistance of Po-1 plants is due not only to "other" loci, but is also due to the Po-1 allele of RPS2. Domain swap

experiments showed that the Po-1 and Col-0 RPS2 promoter sequences had equivalent functionality, and that the coding region for the amino-terminus of Po-1 RPS2 gave a fully functional gene when coupled with the LRR region from Col-0 RPS2 and tested in Po-1 plants. The structural determinant of allele-specific interactions was localized to the LRR-encoding domain of RPS2, where five amino acid differences are predicted between Col-0 and Po-1. Quantitative mapping procedures localized the *avrRpt2*-specific loci to RPS2 and to two other genetic intervals on chromosome 4. The *avrRpm1*-specific loci localized to RPM1 and to a region on chromosome 4 that overlaps with one of the intervals that impacts *avrRpt2*-specific resistance. One or more genes within this interval may be required for function of both the RPS2 and RPM1 resistance pathways.

128 mutants in plant pathogen-signalling
Susanne Berger, Norbert Nass and Dierk Scheel
Leibniz-Institute of plant biochemistry

The response of plants to pathogen attack includes the hypersensitive response, structural changes of the cell wall and accumulation of PR-proteins as well as phytoalexins. Additionally, the expression of a variety of genes is altered. In order to investigate signal transduction events involved in pathogen signalling, mutants of *Arabidopsis thaliana* with altered expression of a pathogen-responsive gene, the *eli3* gene were isolated. The *eli3* gene encodes a dehydrogenase that accepts phenolic aldehydes as substrates. The expression of this gene is inducible by abiotic and biotic stress, such as wounding, pathogens and heavy metals. We used a reporter line containing the *eli3*-promoter fused to β -glucuronidase (GUS) to screen for mutants exhibiting constitutive high GUS activity. Screening of 20000 mutants resulted in 66 mutants showing increased GUS activity. However, in only 6 of these mutants the high GUS activity correlated with increased expression of the endogenous *eli3* gene. In these mutants the expression of several pathogen-responsive genes was characterized. In most cases, expression of PAL and a peroxidase was not elevated indicating that different signalling pathways regulate the expression of these genes and the *eli3* gene. The development of HR/disease after pathogen treatment of these mutants as well as the growth of virulent and avirulent strains of *Pseudomonas syringae* was investigated.

129 The RPP13-Nd disease resistance gene does not require NDR1 or EDS1 and functions independently of salicylic acid accumulation
Peter Bittner-Eddy and Jim Beynon
Horticulture Research International, Wellesbourne, CV35 9EF, UK
We recently cloned a functional RPP13 allele from the *Arabidopsis* accession Nd-1 and showed that it conferred resistance in transgenic Col plants to 5 isolates of the biotrophic oomycete, *Peronospora parasitica* [Bittner-Eddy et al. (2000), *Plant J.* 21, 177-188]. RPP13-Nd encodes an NBS-LRR type protein with a putative amino-terminal leucine zipper (LZ) and shows greatest similarity to RPP8-Ler (28% amino acid similarity), another *Arabidopsis* LZ NBS-LRR protein that confers resistance to *P. parasitica*. Mutational analysis has revealed at least two signalling pathways defined by NDR1 and EDS1 that control pathogen resistance in *Arabidopsis* and that appear to differentially affect R gene proteins having either an LZ or TIR amino-terminal domain, although RPP8-Ler resistance does not exhibit an exclusive requirement for either EDS1 or NDR1

[Aarts et al. (1998), PNAS 95, 10306-10311]. We examined the effect of eds1-1 and ndr1-1 mutations on RPP13-Nd function and found that resistance to any one of the 5 diagnostic *P. parasitica* isolates remained unchanged in our cotyledon based assay indicating no absolute requirement for EDS1 or NDR1 in RPP13-Nd mediated resistance. Mutational analyses have also revealed other Col genes involved in resistance to avirulent *Pseudomonas syringae* pathovars or *P. parasitica* isolates often in a race-specific manner. To examine the effect of these mutations on RPP13-Nd, we constructed genetically uniform lines by crossing Col::RPP13-Nd transgenic plants with these Col mutants and selecting F3 seedlings homozygous for both the transgene and mutation. Macroscopic examination of inoculated cotyledons revealed that RPP13-Nd mediated resistance remained unaltered in pbs2, pad4-1, npr1-1, or rps5-1 mutant backgrounds, yet RPP2 and RPP4 mediated resistance was compromised. Furthermore, we observed no increase in hyphal growth in any of the lines relative to the Col::RPP13-Nd controls when infected tissue was examined under the microscope. In addition, we also found that depletion of salicylic acid (NahG background) had no effect on RPP13-Nd mediated resistance. We conclude that RPP13-Nd operates independently of the salicylic acid signalling pathway and does not depend on NDR1 or EDS1 for function.

130 Knockout of a lipid transfer protein homologue causes programmed cell death and activation of defence responses in *Arabidopsis*

Peter Brodersen*, Morten Petersen*, Anders Bøgh Jensen*, Lise Bolt Jørgensen§, Søren Skov⌘ and John Mundy*

*Department of Plant Physiology, §Department of Evolutionary Botany, ⌘Institute of Medical Microbiology and Immunology, University of Copenhagen, Denmark

We use a Ds gene-trap system [1] to generate insertional mutants and tag genes with potential regulatory functions. One recessive mutant, G376, exhibited run-away cell death at the rosette stage. G376 cell death had apoptotic characteristics, including high levels of reactive oxygen species, dense nuclei, DNA degradation assayed by protoplast FACS analysis, and ectopic expression of the programmed cell death marker SAG13. G376 cell death was apparently not senescence related, as it could not be inhibited by cytokinin, and expression of the SAG12 senescence marker was normal. In contrast, cell death in G376 resembled the hypersensitive cell death normally triggered by avirulent pathogens. First, the mutant constitutively expressed markers for systemic acquired resistance (PR1, PR2, PR5), camalexin biosynthesis (PAD3), and defensive genes such as glutathione S-transferases and peroxidases. Second, the mutant was resistant to infection with normally virulent pathogens. Third, deposition of callose and autofluorescent polyphenolic compounds was observed in G376 cell walls. In addition, G376 homozygotes expressing the NahG salicylate hydroxylase were normal, indicating that salicylic acid is required for cell death in G376. Molecular analysis of the insertion revealed that Ds had created a 30 kb deletion. The mutant phenotype was rescued by a 3.3 kb DNA fragment encoding a lipid transfer protein homologue, but not by any other fragment within the deleted region. These results implicate lipid signaling in the regulation of plant programmed cell death and pathogen responses.

[1] Sundaresan et al. 1995, *Genes & Dev.* 9:1797-1810.

131 Isolation of transposon mutants of *Pseudomonas syringae* pv. *tomato* reduced in virulence on *Arabidopsis thaliana* and *Lycopersicon esculentum*

David M. Brooks¹, Andrew P. Kloek^{1,2}, and Barbara N. Kunkel¹

1. Washington University Department of Biology, St. Louis, Missouri, and 2. Current address: Divergence, LLC, St. Louis, Missouri

We study interactions between the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (Pst), and its hosts, *A. thaliana* and *Lycopersicon esculentum* (tomato). Pst causes severe bacterial speck disease symptoms on both of these plants, thus we are interested in the identification of genes that are involved in pathogenesis, including those that may influence host range. 947 Mini-Tn5 Km-gus transposon mutants were screened by dip inoculation on *A. thaliana* and tomato. Thirty-eight mutants with reduced virulence were isolated. Six of these exhibited a *hrp* mutant phenotype; causing no disease on either host. The remaining 32 exhibited reduced disease symptom formation on both hosts. A genomic sequencing protocol was utilized to determine genomic sequence flanking the Tn5 insertion. Eight mutants possessed a high level of sequence similarity with the coronatine (COR) biosynthetic cluster of *Pseudomonas syringae* pv. *glycinea* PG4180. COR is a bacterial phytotoxin that has been proposed to function as a molecular mimic of methyl jasmonate, a signal molecule in the octadecanoid pathway of plants. Two other mutants appear to define novel loci. Based on inoculation phenotypes, we believe that these COR and novel mutants are impaired in an early step of pathogenesis. For example, disease symptoms increase in severity when the pathogen is injected directly into plant tissues. Also, growth in planta is equivalent to wild-type when inoculated this way. Preliminary data suggests that one of the novel mutants is able to generate COR in culture, while the other does not. Our progress in determining the role of COR in Pst infection using clearly defined insertional mutations in COR biosynthetic genes will be presented.

132 The GCC box mediates jasmonate and pathogen responsiveness in the PDF1.2 promoter of *Arabidopsis*

Rebecca Brown, Kemal Kazan, Don Maclean and John Manners

Co-operative Research Centre for Tropical Plant Protection, University of Queensland, St Lucia, QLD, Australia. 4072

Systemic acquired resistance involves the induction of a range of defence related genes that provide the plant with resistance to a broad-spectrum of pathogenic organisms. The PDF1.2 gene was the first putative defence related gene described for *Arabidopsis* that is activated by pathogen infection via a jasmonate mediated pathway. This is an alternative to the better-studied salicylic acid dependent pathway of defence activation. Analysis of the PDF1.2 promoter has been undertaken in order to determine cis regulatory elements, and thus elucidate upstream signal transduction processes involved in the jasmonate mediated defence response. A series of promoter deletions fused to the *uidA* reporter gene were constructed, and stably introduced into *Arabidopsis*. Second generation transgenic plants were used to examine the role of various promoter regions in systemic and local activation in response to pathogen infection and chemical signal compounds. The promoter contains a GCC box, a motif that has been shown to be important in the regulation of ethylene responsive genes. It seems that this box also mediates jasmonate and pathogen activated signaling in the PDF1.2 promoter. The promoter also contains an

enhancer element approximately 200bp upstream of the GCC box, which significantly increases absolute expression levels, but which does not affect jasmonate induction. Transient bombardment experiments of transcription factors known to bind the GCC box in other systems, into the transgenic reporter plants with and without the GCC motif, are underway. Preliminary results show activation of the GUS reporter via the GCC motif, by at least one of these transcription factors. We are further examining the potentially overlapping roles of these transcription factors in the regulation of jasmonate and ethylene responsive genes.

133 Age-Related Resistance and the SAR-defective mutant, *dir-1*, defective in production of the SAR mobile signal

*Robin K. Cameron, *Julie Kus, *Dan Liu, +Anita Maldonado, oRick Dixon, +Chris Lamb

University of Toronto, Dept. of Botany, 25 Willcocks St, Toronto, Ontario, Canada, M5S 3B2. o The Noble Foundation, Plant Biology Division, 2510 Highway 199 East, Ardmore, OK, USA 73402.+ Sainsbury Lab, John Innes Centre, Colney Lane, Norwich, NR4 7UH, England. Age-Related Resistance and the SAR-defective mutant, *dir-1*, defective in production of the SAR mobile signal. *Robin K. Cameron, *Julie Kus, *Dan Liu, +Anita Maldonado, oRick Dixon, +Chris Lamb. * University of Toronto, Dept. of Botany, 25 Willcocks St, Toronto, Ontario, Canada, M5S 3B2. o The Noble Foundation, Plant Biology Division, 2510 Highway 199 East, Ardmore, OK, USA 73402. +Sainsbury Lab, John Innes Centre, Colney Lane, Norwich, NR4 7UH, England. Systemic Acquired Resistance (SAR) is induced by an initial "immunizing" infection in one part of the plant which produces broad non-specific resistance throughout the plant to normally virulent pathogens. We have identified a SAR-defective mutant *dir-1* (defective in induced resistance) that is defective only in the SAR pathway. Recent studies (using petiole exudates) indicate that the *dir-1* mutant does not make the SAR mobile signal. It is possible that the *DIR-1* gene codes for a protein which is itself the phloem mobile signal or it may be involved in producing the signal molecule. Studies that address this question will be presented. During the course of studying *dir-1* it was observed that older plants became more resistant to normally virulent *Pseudomonas syringae* pv *tomato*. We began to study this Age-related resistance (ARR) phenomenon to determine when and why it happens in order to avoid it during SAR experiments. ARR occurs in SAR-defective mutants and is not associated with PR-1 expression. Preliminary evidence suggests that a bactericidal compound is produced in the intercellular space in these older plants only in response to virulent infection. NahG plants which cannot accumulate SA do not exhibit ARR suggesting that the bactericidal compound produced in plants exhibiting ARR may be phenolic in nature. We are currently attempting to identify this compound which is also affective in vitro against the intercellular fungus *Cladosporium*.

134 A genetic screen for *Arabidopsis* mutants that are insensitive to *avrRpt2* virulence activity

Zhongying Chen and Barbara N. Kunkel
Washington University

The *avrRpt2* gene was initially cloned from the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Pst) as an avirulence gene. It encodes a factor that can be detected by

Arabidopsis plants carrying a functional RPS2 gene, resulting in the activation of plant defense responses. With the onset of such "gene-for-gene" resistance, the virulence of Pst strain DC3000 carrying *avrRpt2* on RPS2 plants is dramatically reduced. We recently demonstrated that, on Arabidopsis plants lacking a functional RPS2 gene (*rps2* mutants), the *AvrRpt2* protein promotes virulence of PstDC3000, either when delivered by the bacterial pathogen during infection or when expressed in transgenic *rps2* plants. One example of *avrRpt2* virulence activity is that *AvrRpt2* can specifically interfere with RPM1-mediated resistance. Accordingly, *avrRpt2* transgenic plants carrying a functional RPM1 gene are susceptible to infection by PstDC3000 carrying *avrRpm1*, a gene that normally triggers gene-for-gene resistance on RPM1 plants. Based on this observation, we EMS mutagenized the seeds of *rps2* plants transgenically expressing *AvrRpt2*, and challenged the M2 plants with PstDC3000(*avrRpm1*), then looked for mutants that are resistant due to a restoration of RPM1-mediated gene-for-gene resistance. To date, we have screened ~50,000 M2 plants and have identified 12 putative mutants. We will present data describing the initial characterization of several of these putative mutants.

135 A Examination of SA, JA and Ethylene Mediated Disease Resistance Through Epistasis Analyses

Joseph D. Clarke, Heidi Ledford, and Xinnian Dong

Duke University, DCMB/Botany, LSRC Building, Research Drive, Durham, NC 27708-1000

Disease resistance in Arabidopsis is comprised of multiple signal transduction pathways regulated by phytohormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). Epistasis analyses between mutants that convey a resistance-altered phenotype have been indispensable in identifying and characterizing these pathways. Here we report some conclusions of an epistasis study designed to explore the relationship between the NPR1-independent and JA/ET-mediated resistance responses induced by *cpr5* and *cpr6*. Using the SA-deficient mutant *eds5*, the ET-insensitive mutant *ein2*, and the JA-unresponsive mutant *jar1*, we show that disease resistance is a function of the expression of both SA- and JA/ET-mediated pathways. Our data show that *cpr5* and *cpr6* regulate resistance through distinct pathways and that SA-mediated, NPR1-independent and NPR1-dependent resistance works synergistically with components of the JA/ET-mediated resistance response. A model is presented based on the resistance profiles obtained from the double and triple mutants.

136 FINE-SCALE MAPPING OF RPP7, A GENE CONFERRING NON-SALICYLIC ACID DEPENDENT DOWNY MILDEW RESISTANCE IN ARABIDOPSIS

Cuzick, A., Can, C., Williams, K., Beynon, J. L., and Holub, E. B.

Horticulture Research International, Wellesbourne, Warwick. CV35 9EF, UK

The gene RPP7 in Columbia (Col-0) Arabidopsis confers resistance to *Peronospora parasitica* (isolate Hiks1), the biotrophic oomycete which causes downy mildew. This source of resistance is of interest as an example that appears to involve a non-salicylic acid dependent defence response. This as yet undescribed defence response also appears to be independent of EDS1 and NDR1 expression; two signalling genes which have been proposed to distinguish between responses conferred by different classes of receptor-like

resistance genes, TIR-NBS-LRR and LZ-NBS-LRR respectively (Aarts et al., PNAS 95, 10306-10311). It also appears to be unaffected by mutations in the ethylene or jasmonic acid response pathways (EIN2, JAR1, and COI1). Therefore molecular characterisation of RPP7 is important to define an example of a potential receptor for a novel defence pathway. RPP7 has been mapped to a location on the bottom of chromosome 1, using 200 recombinant inbred lines at F9 from a cross between accessions Col-0 and Nd-1. A contig of IGF BACs is available across the RPP7 map interval, however only one of these has currently been sequenced to date. Markers have been generated from these BACs to position the locus to a region spanned by 2 BACs (100-200kb), with 3 recombinant lines remaining. Col-rpp7 mutants have been selected and confirmed genetically including 1 EMS treated and 4 fast neutron treated. At least one fast neutron mutant shows a band shift, compared to wild-type, when probed with an R-gene containing subclone from one of the spanning BACs. Several hybridising bands appeared using this probe indicating numerous R-gene like sequences within this area. Further marker development will be attempted to narrow down the interval to one BAC using remaining recombinants. Cosmids will be produced from it to transform into the mutant lines and screen for resistance to Hiks1.

137 Characterization of *rrsI*, a novel *Arabidopsis thaliana* gene conferring resistance to different strains of the bacterial pathogen, *Ralstonia solanacearum*

Deslandes L.1, Olivier J.1., Holub E. 2, Bittner-Eddy P. 2, Beynon J. 2 and Marco Y.1
1Laboratoire de Biologie Moléculaire des Relations Plantes/Micro-organismes CNRS-INRA, BP 27, 31326 Castanet-Tolosan, France

The interactions between *Arabidopsis thaliana* and various strains of *Ralstonia solanacearum*, a soil-borne vascular pathogen found mostly in tropical and subtropical areas, were examined. By root inoculation, a wild type strain, GMI1000 wilted the ecotype Col-5 in an *hrp*-dependent process whereas another *Arabidopsis* ecotype, Nd-1, was found to be resistant to most strains of *R. solanacearum* including strain GMI1000. Genetic analysis of segregating populations generated from crosses between susceptible and resistant ecotypes indicated that a single recessive locus in Nd-1 confers resistance to strain GMI1000. F8 recombinant inbred lines generated between these ecotypes Col-5 and Nd-1 were used to map this disease resistance locus, *RRS1*, on chromosome V. The positional cloning of *RRSI* was performed and led to the isolation of a novel resistance gene. The structure of this gene as well as results obtained by complementation experiments will be presented.

138 Molecular genetic analysis of a novel lesion mimic mutant of

Sendil Devadas and Ramesh Raina

Inter-college Graduate Program in Plant Physiology and Department of Biology, Penn state University, University Park, PA 16802. email:skd4@psu.edu

Hypersensitive response (HR) is an active host cell suicide mechanism displayed by resistant plants against incompatible pathogens. Several lesion mimic mutants have been identified and analyzed in *Arabidopsis* that show HR-like cell death in the absence of any pathogen. Here we describe the isolation and characterization of *hrl1* (hypersensitive response like lesions) that exhibits spontaneous cell death accompanied by various cellular markers of HR. Northern analysis indicate that both SA-dependent SAR genes

and ethylene/JA-dependent defense genes are induced to very high levels. In addition, SA and ethylene levels are elevated up to 5-fold in *hrl1*. Furthermore, growth of virulent bacterial and fungal pathogens are significantly reduced in *hrl1* compared to the wild type. Epistasis analyses of *hrl1npr1* indicate that PDF1.2 expression in *hrl1* is partially dependent on NPR1. Removal of SA in *hrl1* results in the loss of PR gene expression and interestingly the punctate nature of lesions seen in *hrl1* become diffused, similar to the lesions of 'spreading class' lesion-mimic mutants. Furthermore, preliminary analysis of *hrl1etr1* indicates that PR-1 expression is reduced in *hrl1etr1*, suggesting an additional role for ethylene in regulating PR-1 expression in *hrl1*. Expression of *AtrbohD*, a homolog of the major subunit (*gp91phox*) of the mammalian NAD(P)H oxidase complex, is induced to high levels in *hrl1*. This observation suggests that HRL1 may negatively regulate ROI production and this aberrant regulation might be responsible for the lesion phenotype and other cellular responses in *hrl1*. The recessive *hrl1* mutation has been mapped to the bottom arm of chromosome IV within 1 cM interval near *RPS2* gene. Further cloning and characterization of *hrl1* will enable us to explore the labyrinth of signal transduction pathways underlying the multiple defense responses in plants. Results of these studies and other experiments will be presented.

139 Molecular and Functional Study of NPR1 and AHBP-1b Interaction in Arabidopsis

Weihua Fan, Yuelin Zhang and Xinnian Dong

DCMB Group, Dept. Botany, Po Box 91000, Duke University, Durham, NC 27708

NPR1 is a key regulator for gene expression in the onset of SAR. Recently, we have cloned an NPR1-interacting protein, NIF1, from tomato through yeast two-hybrid screen with Arabidopsis NPR1 as bait. We also found that Arabidopsis homologues of NIF1, AHBP-1b (TGA2), TGA3, OBF5 (TGA5) and TGA6 interact with NPR1 both in yeast and in vitro. In addition, these transcription factors were found to bind a SA-responsive element in PR-1 gene promoter in gel mobility shift assay. Current work is focused on studying the in vivo interaction between NPR1 and the TGA family transcription factors, and their function in plant defense. In addition to screening for insertional mutants of the TGA family of transcription factors, transgenic plants expressing the TGA factors were generated. Preliminary characterization of these transgenic plants showed no alteration in PR-1 gene expression. Other aspects of plant defense are currently being tested. Another approach is to use dominant-negative forms of TGA factors to study its function and in vivo interaction with NPR1. DNA-binding and NPR1-interacting activity of the dominant-negatives has been tested before transformation into wildtype Arabidopsis plants. Initial characterization of transgenic plants did show expected dominant-negative effect. More molecular and biochemical experiments are underway to further characterize the in vivo interaction between AHBP-1b and NPR1, and the results will be discussed.

140 Isolation of novel Arabidopsis mutants with altered defensin expression

Simone Ferrari, Timothy P. Durrett, Julie M. Stone, Felice Cervone¹, Giulia De Lorenzo¹, Frederick M. Ausubel

Massachusetts General Hospital - Department of Molecular Biology, Boston, MA (USA);
¹Universita' degli Studi "La Sapienza" - Dipartimento di Biologia Vegetale, Roma (Italy)

Defensins are small antimicrobial polypeptides produced by animals and plants after microbial infections. Whereas other plant pathogenesis-related proteins, like PR1, are induced by salicylic acid (SA), defensin expression requires the concomitant activation of the jasmonic acid (JA) and the ethylene pathways. Increasing evidence, however, suggests the existence of a cross-talk between the two pathways. Since most of the available data about the regulation of defensin expression come from mutants isolated for alterations in the SA-dependent responses, we attempted a genetic screening to identify genes that specifically regulate defensin expression.

The fungal toxin fumonisin B1 (FB1) was used in a pathogen-free screening for Arabidopsis mutants with altered expression of defensins; two lines were selected for further analysis. The *dde1* (deficient defensin expression 1) mutation causes defective expression of defensins and PR1 after treatment with FB1, and confers enhanced resistance to a fungal pathogen. The *cde1* (constitutive defensin expression 1) mutant displays high basal levels of defensin, and exogenous SA and JA abolish this constitutive expression. A database search revealed that the Arabidopsis genome encodes a family of closely related defensin genes, whose expression is differentially regulated in seedlings and adult plants. In *dde1* plants, none of these genes was highly induced after FB1 treatment.

141 Tomato Allene Oxide Synthase and Fatty Acid Hydroperoxide Lyase, Two Cytochrome P450s Involved in Oxylin Metabolism, are Targeted to Different Membranes of Chloroplast Envelope

John E. Froehlich, Ayah Itoh, and Gregg A. Howe

DOE-Plant Research Laboratory

Allene oxide synthase (AOS) and hydroperoxide lyase (HPL) are related cytochrome P450s (CYP74) that metabolize a common fatty acid hydroperoxide substrate to different classes of bioactive oxylin within the chloroplast. Here, we report the use of *in vitro* import assays to investigate the targeting of tomato AOS (LeAOS) and HPL (LeHPL) to isolated chloroplasts. LeAOS, which contains a typical N-terminal transit peptide, was targeted to the inner envelope membrane by a route that requires both ATP and proteinase-sensitive components on the surface of the chloroplast. Imported LeAOS was peripherally associated with the inner envelope, with the bulk of the protein oriented toward the stroma. LeHPL, which lacked a typical chloroplast-targeting sequence, was targeted to the outer envelope by an ATP-independent and protease-insensitive pathway. Imported LeHPL was integrally associated with the outer envelope, with most of the protein exposed to the intermembrane space. We conclude that LeAOS and LeHPL are routed to different envelope membranes by distinct targeting pathways. Partitioning of AOS and HPL to different envelope membranes may provide a mechanism to maintain the functional autonomy of two oxylin pathways that metabolize the same hydroperoxide substrate.

142 Identification of an Arabidopsis locus involved in the recognition of a disease-specific protein from *Erwinia amylovora*

Eric R. Garr and Steven V. Beer

Department of Plant pathology, Cornell University, Ithaca, NY 14853, USA

The disease-specific gene *dspE* of *Erwinia amylovora* is absolutely required for the bacterium to cause the fire blight disease of apple and pear. *dspE* is a homolog of *avrE*, an avirulence gene of *Pseudomonas syringae* pv. *tomato*. Like *avrE*, *dspE* renders *P. syringae* pv. *glycinea* avirulent on several cultivars of soybean. We are using *Arabidopsis thaliana* to study the avirulence mechanisms of *dspE*. *Escherichia coli* strain MC4100 containing a functional *E. amylovora* *hrp* (hypersensitive response and pathogenicity) gene cluster is capable of delivering the *dspE* gene product to plant cells. Infiltration of MC4100/*hrp/dspE* into the apoplast results in elicitation of the hypersensitive response (HR) in several ecotypes of *Arabidopsis* including the common ecotype Landsburg-Erecta (Ler), but not in Columbia-g11 (Col-g11). Agrobacterium-mediated transformation of Ler with a gene construct expressing *dspE* under the control of the CAMV 35S promoter yielded few transformants that died shortly after true leaf initiation. These plants typically displayed small necrotic lesions and abnormal leaf phenotypes. Transgenic plants expressing *dspE* from a dexamethasone-inducible promoter are being generated to study the effect of *dspE*-induced cell death in *Arabidopsis*. Translocation of *DspE* to plant cells by MC4100/*hrp/dspE* results in cellular responses typical of the HR; namely rapid expression of pathogenesis-related (PR) gene signals and electrolyte leakage. Genetic analysis of the *dspE*-elicited HR phenotype demonstrated that it is conditioned by a single dominant locus that is strongly influenced by genetic background. The locus is being mapped and we hope to isolate the gene responsible using a positional cloning approach.

143 Epistasis Analysis of *pad4* and Other Mutations Affecting Salicylic Acid Signaling

Jane Glazebrook¹, Dayadevi Jirage^{1,2}, Nan Zhou², Bret Cooper¹, Joseph D. Clarke³, I-ching Yu⁴, Xinnian Dong³ and Andrew F. Bent⁴

¹Novartis Agricultural Discovery Institute, Inc, ²University of Maryland Biotechnology Institute, ³Duke University, ⁴University of Illinois at Urbana-Champaign

Mutations in *PAD4* partially block salicylic acid (SA)-dependent signaling after infection with *Pseudomonas syringae*. Mutant plants exhibit reduced SA levels, reduced PR-1 expression, and reduced levels of camalexin after *P. syringae* infection. They also display enhanced susceptibility to *P. syringae* and *Peronospora parasitica*. In contrast, mutations in *cpr1*, *cpr5*, *cpr6*, *dnd1*, and *dnd2* cause constitutively high levels of SA and PR-1 mRNA, and resistance to *P. syringae*. In an effort to understand the hierarchy of components of the SA signal transduction pathway, *cpr1 pad4*, *cpr5 pad4*, *cpr6 pad4*, *dnd1 pad4*, and *dnd2 pad4* double mutants were constructed and characterized.

Phenotypes studied included camalexin production, constitutive and inducible expression of PR-1, *PAD4*, and PDF1.2, dwarfism, and susceptibility to *P. syringae* and *P. parasitica*. All phenotypes of *cpr1* plants were suppressed by *pad4*, suggesting that *CPR1* acts upstream from *PAD4* in the signal transduction network. The phenotypes of the other double mutant combinations were complex, with some phenotypes resembling *pad4* and some resembling the constitutive mutant. Curiously, of the three lesion-mimic mutants *cpr5*, *dnd1*, and *dnd2* (*dnd1* and *dnd2* displayed lesion-mimic phenotypes under our growth conditions), *cpr5* and *dnd2* partially suppressed the camalexin-deficient phenotype of *pad4*. Taken together with our previous observation that *pad4* is not required for camalexin production after infection by bacterial strains carrying *avrRpt2*,

this suggests that the lesions in *cpr5* and *dnd2* plants may qualitatively resemble those triggered by *avrRpt2*, while the lesions in *dnd1* plants may be qualitatively different. Constitutive expression of PR-1 was at least partly suppressed by *pad4* in all of the double mutant plants, as was *P. syringae*-induced expression of PR-1, indicating that PR-1 expression in all of these constitutive mutants occurs in a PAD4-dependent manner.

144 Global expression analysis of genes differentially expressed during activation of HR and SAR in *Arabidopsis thaliana*

Ana Maria Gomez-Buitrago, Lynn Lindstrom, Nina Fedoroff and Ramesh Raina
Biology Department and The Biotechnology Institute, Pennsylvania State University,
State College, PA 16802, USA

During incompatible interactions, plants induce hypersensitive response (HR) which in many cases is followed by activation of systemic acquired resistance (SAR). SAR can also be induced in plants by exogenous application of salicylic acid (SA). In order to identify the plant genes involved in activation of HR and SAR, we have used PCR-based Suppression Subtractive Hybridization (SSH) approach to construct cDNA libraries representing *Arabidopsis* (ecotype Col-0) genes differentially expressed in response to avirulent pathogen (*Pst* DC3000 expressing *avrRpm1*) and SA treatments. Analysis of 500 clones from SA-subtracted library identified 175 clones representing unique genes. Expression analysis by traditional northern revealed that among the clones tested, most (73%) are induced in response to pathogen and/or SA treatment. For about 34% of the unique genes, no EST matches could be found. Analysis of ~700 clones from library representing genes induced in response to avirulent bacterial pathogen identified 319 unique genes. For ~38% of these genes, no EST matches could be found. Based on the homology to the known genes, functions could be assigned to ~75% of the unique genes identified in SA and avirulent pathogen libraries and were predicted to encode proteins with a variety of functions such as signal transduction proteins, transcription factors, biochemical enzymes etc. Rest of the 25% genes had no significant homology to any gene in the public database. The clones from these libraries were used to construct microarrays on silanized glass slides using a robot. For detailed temporal expression analysis, these DNA microarrays were probed with fluorescently labeled cDNAs made from total RNA isolated from plants at different time intervals after treatment with SA or pathogen and corresponding controls. The microarrays were scanned and analyzed using the Imagen software (Biodiscovery). These studies should help us better understand the molecular events involved in activation of defense in plants against pathogens and to identifying the target genes for developing transgenic plants with enhanced resistance to pathogens.

145 Analysis of RPP1: a functional resistance gene from *Arabidopsis*

Anna Gordon, Peter Bittner-Eddy, Elly Speulman¹, Eric Holub, Jim Beynon
Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK ¹Present
Address: Department of Molecular Biology, CPRO-DLO, PO Box 16, 6700 AA
Wageningen, The Netherlands

In the *Arabidopsis* accession Niederzenz (Nd-1), resistance to three *Peronospora parasitica* (downy mildew) isolates maps to the RPP1 (Recognition of *P. parasitica*) resistance locus located on the bottom arm of Chromosome 3. Genomic clones that

contain R gene sequence mapping to the RPP1 locus in Nd-1 have been sequenced and these have been used to transform susceptible plants. Genes at this locus code for functional proteins that are capable of recognizing several different pathogen isolates and one of the genes is capable of recognizing all three *P. parasitica* isolates. The sequence analysis is revealing a complex picture. There are at least four full-length copies of the RPP1 gene in the Nd-1 accession. However, the presence of truncated forms of the gene and retrotransposon sequences suggests that gene duplication and inactivation has occurred at this locus. Within the putative translation products of the full-length genes there is a high degree of amino acid sequence conservation, suggesting that these genes are indeed paralogs. The RPP1 genes are members of the TIR-NBS-LRR family of R genes. Analysis of gene structure reveals that most variation between paralogs lies within the leucine rich repeat domains. Work presented here will describe the evolution of the RPP1 locus at both a sequence and a locus level.

146 *Arabidopsis thaliana* EDS4 Contributes to Salicylic Acid (SA) Dependent Expression of Defense Responses : Evidence for Inhibition of Jasmonic Acid Signaling by SA

Vaijayanti Gupta^{a,c}, Michael G. Willits^b and Jane Glazebrook^c,
^aMolecular and Cell Biology Graduate Program, Department of Microbiology and Immunology, University of Maryland, College Park, MD 20742, USA., ^bNovartis Agribusiness Biotechnology Research Inc., P.O. Box 12257, 3054 Cornwallis Road, Research Triangle Park, NC 27709-2257, USA., ^cNovartis Agriculture
The *Arabidopsis* enhanced disease susceptibility 4 (*eds4*) mutation causes enhanced susceptibility to infection by the bacterial pathogen *Pseudomonas syringae* pv. *maculicola* ES4326 (Psm ES4326). Gene-for-gene resistance to bacteria carrying the avirulence gene *avrRpt2* is not significantly affected by *eds4*. Plants homozygous for *eds4* exhibit reduced expression of the pathogenesis-related gene PR-1 after infection by Psm ES4326, weakened responses to treatment with the signal molecule salicylic acid (SA), impairment of the systemic acquired resistance response, and reduced accumulation of SA after infection with Psm ES4326. These phenotypes indicate that EDS4 plays a role in SA-dependent signaling. SA has been shown to have a negative effect on activation of gene expression by the signal molecule jasmonic acid (JA). Two mutations that cause reduced SA levels, *eds4* and *pad4*, cause heightened responses to inducers of JA-dependent gene expression, providing genetic evidence to support the idea that SA interferes with JA-dependent signaling. Two possible working models of the role of EDS4 in governing activation of defense responses are presented.

147 TURNIP CRINKLE VIRUS SUPPRESSION OF THE HYPERSENSITIVE RESPONSE

Thomas M. Hammond and Kristin K. Wobbe
Worcester Polytechnic Institute, Worcester, MA 01609
Turnip crinkle virus elicits an active resistance response, including hypersensitive response (HR) induction and PR gene expression, when inoculated on Di-17 *Arabidopsis*. However, a percentage of HR-forming Di-17s eventually display signs of systemic infection. Interestingly, symptomatic leaves of these plants fail to display macro-lesions even though they contain both the *avr* of TCV and high levels of PR-1 mRNA. Trypan

blue staining of the same leaves demonstrates a fascinating pattern of cell death with symptomatic leaves frequently showing micro-lesions lining the vasculature, primarily at the base of the leaf. In situ hybridization with a TCV-specific probe suggests that virus is present in a substantial portion of the vasculature in both symptomatic and asymptomatic leaves. Furthermore, the TCV-specific probe hybridizes to large amounts of viable leaf tissue in symptomatic leaves indicating that the presence of the virus does not necessarily correlate with cell death. Additionally, while asymptomatic leaves respond to TCV re-inoculation with an HR, symptomatic tissue does not form visible lesions upon re-inoculation. These results suggest that TCV has the ability to suppress or avoid triggering the HR.

148 Expression profiling of host responses to viral infection

Y.-M. Hou, S. Quan, H.-S. Chang, T. Zhu and S. Whitham

Novartis Agricultural Discovery Institute, Inc., 3115 Merryfield Row, San Diego, CA 92121 USA

To characterize global changes in host gene expression during virus infection and to understand the molecular mechanisms involved in host-virus interactions, our group utilizes the oligo-based microarray technique. Arabidopsis plants will be inoculated with representatives from the cucumovirus, tobamovirus, tobnavirus, potyvirus, and geminivirus groups, and examined for their gene expression profile at different infection stages. Results from this study will identify commonly induced or suppressed genes from different virus treatments and specific genes related to infection of each virus group and to infection of plant RNA vs. DNA viruses. These genes will be primary targets for reverse genetic analysis to characterize their function and role in virus infection. In addition, the correlation of these host genes on viral pathogenicity and various symptom phenotypes may be revealed. By gaining knowledge in this area we will be able to better understand the molecular mechanisms of plant-virus interaction.

149 CAULIFLOWER MOSAIC VIRUS MOVEMENT PROTEIN INTERACTS WITH A COMPONENT OF THE CELL TRAFFICKING MACHINERY

Zhong Huang, Vyacheslav M. Andrianov, Yu Han, and Stephen H. Howell

Boyce Thompson Institute, Cornell University, Ithaca, NY 14853, USA

Gene I of cauliflower mosaic virus (CaMV) encodes a protein that is required for virus movement. The CaMV movement protein (MP) was used in a yeast 2-hybrid system to screen an Arabidopsis cDNA library for cDNAs encoding MP interacting proteins (MPIPs). Three different clones were found encoding proteins (MPI1, -2 and -7) that interact with the N-terminal third of the CaMV MP. The interaction between the CaMV MP and MPIP7 appeared to be significant because the interaction was influenced by MP mutants known to affect virus infectivity. For example, a non-infectious mutant, ER2A, with two amino acid changes in the N-terminus of the MP failed to interact with MPI7, while a MP from an infectious second-site mutant, which differed from ER2A by only a single amino acid change, interacted positively. MPI7 is expressed widely in Arabidopsis plants and is related in sequence, size and hydrophathy profile to a rat protein (PRA1) that associates with the vesicle transport machinery in mammalian cells. MPI7 was tagged with GFP and the resulting MPI7:GFP fusion was found to associate with subcellular particles in plant cells. The subcellular particles undergo directed movement when

viewed by time lapse photography. These findings are consistent with a presumed role of the CaMV MP in viral movement and suggest that the viral MP may interact with a component of the trafficking machinery in plants.

150 Identification of a promoter region involved in BCTV early-gene expression
Jingyung Hur and Keith R. Davis
The Ohio State University, 207A Biotech Center, 1060 Carmack rd. Columbus, OH
43210

Beet Curly Top Virus (BCTV) is a ssDNA virus which has small genome of 3kb. This virus contains only seven open reading frames (ORFs) which encode viral proteins essential for the virus life cycles. For a significant portion of its life cycle, the virus is dependent on host enzymes to replicate and express its genes in plants. Thus, this virus can provide a good system to understand the mechanisms of gene expression in plant cells. Of the seven viral ORFs, L1 (also known as C1) is known to encode a viral protein involved in initiation of viral replication and is believed to be the first viral protein to be expressed in virus infected plant cells. In this study, we identified promoter regions of viral ORF L1 using reporter gene fusions expressed in transgenic Arabidopsis. Promoter elements for this early viral gene reside not only in the common intergenic region but also in the viral protein coding region. The promoter activity of L1 in transgenic plants was confined to vascular tissues. This localization is consistent with virus localization in virus infected plants - BCTV is a phloem limited virus.

151 Characterization of *ups1*, an Arabidopsis thaliana mutant defective in the induction of defense responses following infection by *Pseudomonas syringae* pv *maculicola*
Laure J.M. Jason and Katherine J. Denby
Department of Biochemistry, University of Cape Town, Private Bag Rondebosch 7701
South Africa

The focus of this research is on characterizing plant signaling pathways activated in response to phytopathogen attack. In Arabidopsis thaliana, infection by *Pseudomonas syringae* pv *maculicola* leads to increased expression of the tryptophan (*trp*) biosynthetic enzymes and concomitant accumulation of the phytoalexin camalexin, a secondary metabolite of the tryptophan pathway. In order to identify candidate genes involved in this signal pathway, Arabidopsis mutants defective in the induction of *trp* biosynthetic genes following *Pseudomonas* infection were isolated. These mutants were also defective in the induction of the *trp* enzymes under various abiotic stress conditions and were named *ups* (underinducer for pathogen and stress). Following *Pseudomonas* infection, plants homozygous for the monogenic, recessive mutation *ups1* accumulated significantly reduced levels of camalexin and were defective in the induction of glutathione S-transferase when compared to wild type plants. Preliminary data suggest that *ups1* hinders the induction of the defensin PDF1.2 and pathogenesis related (PR) protein PR2 following pathogen attack. Pathogen growth was less restricted in *ups1* plants than in wild type plants suggesting that this signaling defect has an effect on pathogenesis. Salicylic acid and jasmonic acid are known intermediates in signaling pathways leading to the expression of selected PR proteins and defensins. The expression of these genes following exogenous application of salicylic acid and methyl jasmonate was monitored in

ups1 and wild type plants to investigate the position of UPS1 action relative to known signaling components. Complementation and double mutant analysis is being used to establish allelic and epistatic relationships between ups1 plants, other ups mutants and known signaling mutants. Linkage has been established between ups1 and molecular chromosomal markers as a first step towards cloning UPS1 using a map-based approach.

152 Cloning and characterization of Arabidopsis NPR1-homologous genes from apple (*Malus pumila*)

Qiaoling Jin, Patrick Hart, Alan Jones, and Sheng Yang He

Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI48824

The Arabidopsis thaliana NPR1 gene (nonexpressor of pathogenesis-related genes, also called NIM1) is a key regulator of systemic acquired resistance (SAR) against a broad spectrum of pathogens. The *npr1* mutant is impaired in the ability to mount a SAR response even after treatment with salicylic acid (SA) or benzothiadiazole (BTH). Overexpression of NPR1 confers enhanced resistance to multiple pathogens. To determine whether an NPR1 ortholog exists in apple, and whether this gene can be used to enhance resistance against the devastating apple fire blight pathogen *Erwinia amylovora*, we screened an apple cDNA library constructed from apple seedlings treated with BTH. The coding region of the *A. thaliana* NPR1 gene was used as a probe in this screen. From 2 million plaques, we obtained 10 positive clones that were subsequently classified into 3 groups based on restriction enzyme analysis. Three representative clones were sequenced and designated MpNPR1-1, MpNPR1-2 and MpNPR1-3, respectively. The deduced amino acid sequence of MpNPR1-1 had 72% identity to that of MpNPR1-2 and 90% identity to that of MpNPR1-3. All of them shared about 35% amino acid identity with the *A. thaliana* NPR1. RNA blot analysis indicated that only MpNPR1-1 was induced in apple seedlings treated with BTH. Overexpression of MpNPR1-1 in the *A. thaliana npr1* mutant partly recovered the PR-1 gene induction upon BTH treatment, suggesting that MpNPR1-1 is likely to be an apple ortholog of the *A. thaliana* NPR1 gene. This work provides the basis for future genetic engineering of disease resistance in apple and possibly other pome fruits.

153 Genes expressed in planta by the plant pathogen *Pseudomonas syringae* during infection of *Arabidopsis thaliana*

Vinita Joardar, Lisa Y.Z. Gao, Jens Boch and Barbara N. Kunkel

Department of Biology, Washington University, St. Louis, MO

Plant pathogenic bacteria are specifically adapted to grow inside the plant host. We are interested in studying virulence factors of the pathogen *Pseudomonas syringae* pv. *tomato* that are involved in colonization of its host *Arabidopsis thaliana*. An *in vivo* selection (IVET) screen yielded several genes that are expressed in planta during the infection process. The IVET fusions were sequenced to determine the identity of the plant-specific promoters. As expected, the screen detected genes homologous to known virulence factors, *avr* genes, and stress-related genes. Moreover, many novel genes were identified. These genes either have no homology to known genes or have not been shown to function as virulence factors. Expression of a subset of these genes requires the sigma factor HrpL, suggesting that these genes encode target proteins of the Type III secretion

system. We present the analysis of some of the novel genes, and the preliminary results of a reverse genetics approach to explore their role in pathogen virulence.

154 GeneChip Analysis of Disease Resistance Responses

Fumiaki Katagiri, Wenqiong Chen, Yi Tao, Jane Glazebrook, Hur-Song Chang, Bin Han, Tong Zhu, and Guangzhou Zhou

Novartis Agricultural Discovery Institute, Inc., 3115 Merryfield Row, San Diego, CA, 92121

The Novartis Agricultural Discovery Institute, Inc (NADII) and Affymetrix have collaborated and produced a GeneChip for Arabidopsis. This chip represents approximately 8,700 Arabidopsis genes. We have used this chip to study changes in gene expression that occur after pathogen attack. In one set of experiments, we studied gene expression changes associated with gene-for-gene resistance mediated by the R gene RPS2 and the cognate bacterial avirulence gene *avrRpt2*. Wild-type plants were infected with a high dose of bacteria carrying or lacking *avrRpt2*, and bacteria carrying *avrRpt2* were used to infect wild type and *rps2* mutant plants. Samples were collected six hours after infection. These experiments all used plants of the Columbia ecotype. A few experiments were also performed using plants of the Ws ecotype. Large differences between the responses of the two ecotypes were observed. In another set of experiments, we studied gene expression changes associated with mutations that affect local salicylic acid signaling. Wild type and mutant plants were infected with a low dose of the virulent pathogen *Pseudomonas syringae* pv. *maculicola* ES4326 or mock infected, and sampled 30 hours after infection. Clustering analysis of gene expression patterns, used to arrange the signaling genes defined by the mutations into a hierarchy that may represent their sites of action in the salicylic acid signal transduction network, and additional detailed analysis will be presented.

155 Arabidopsis homologue of a Gene Encoding a Putative NADPH oxidase from Parsley

Magdalena Krzymowska, Markus Tschöpe, Heidi Zinecker and Dierk Scheel

Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle, Germany

Pathogen recognition by plants triggers a multicomponent defense response. Early processes comprise changes of the ion permeability of the plasma membrane, protein phosphorylation/dephosphorylation and the production of reactive oxygen species (ROS), referred to as oxidative burst. ROS play a role in several aspects of the plant's response to pathogen attack. A growing body of evidence suggests that generation of ROS upon pathogen attack is mediated by an NAD(P)H-oxidase located in the plasma membrane. As the first step the enzyme forms superoxide radicals which are then converted to oxygen and hydrogen peroxide either spontaneously or by an extracellular superoxide dismutase. The presence of an oxidase that catalyzes NADPH-dependent production of O₂⁻ was demonstrated in cells and microsomal fractions of several plants. However, no plant NADPH-oxidase has been isolated so far.

In the present study we have purified an enzyme with NADPH-oxidase activity from parsley cells. In contrast to the mammalian NADPH-oxidase involved in production of ROS in phagocytes, the parsley enzyme apparently exists in monomeric form with a molecular mass of approximately 100 kDa. Partial amino-acid sequence enabled us to

clone two corresponding genes. Several heterologous expression systems were employed to demonstrate enzymatic activity of the recombinant protein. These experiments are still in progress. Expression analysis revealed no activation of the gene at the transcriptional level after elicitor treatment of parsley cells. However, the protein was present in all organs of untreated plants. Peptide antibodies detected the protein in a plasma membrane-enriched cell fraction.

Search of *Arabidopsis thaliana* data bases resulted in the detection of a highly homologous sequence. Moreover, the properties of the predicted *Arabidopsis* protein are similar to the parsley one. Thus cloning of the cDNA opened the perspective to transfer the studies to *A. thaliana*. *Agrobacterium* -mediated plant transformation was performed to generate plants with altered levels of the putative NADPH-oxidase. Characterization of the transgenic plants could answer the question whether the protein is involved in the defense response of *Arabidopsis*.

156 Analysis of resistance to *Pseudomonas syringae* in a coronatine insensitive (*coi1*) mutant of *Arabidopsis*

Barbara N. Kunkel¹, Shashi Sharma², Daniel F. Klessig², James Schoelz³, John Vogel⁴, and Andrew P. Kloek¹

¹Washington University, St. Louis; ²Waksman Inst. Rutgers, The State University of New Jersey, Piscataway, NJ; ³University of Missouri, Columbia, MO; ⁴ Carnegie Inst. of Washington, Stanford, CA

We are interested in identifying and characterizing *Arabidopsis* genes that are involved in mediating responses to pathogen attack. In a screen for mutants with enhanced resistance to the bacterial pathogen *Pseudomonas syringae* pv. tomato (Pst) we isolated a mutant, *coi1-2*, that maps to the Coronatine insensitive (COI1) locus. *coi1* mutants are insensitive to the plant hormone methyl jasmonate (JA) and the bacterial phytotoxin coronatine, which has been proposed to act as a molecular mimic of JA. *coi1* mutant plants are male sterile and are resistant to several virulent pathovars of *P. syringae*. Conversely, *coi1* mutants are not resistant to virulent fungal (Erysiphe) or viral (CaMV) pathogens. We are interested in understanding the molecular basis of resistance to *P. syringae* in the *coi1* mutant. Since the JA-dependent defense pathway is blocked in *coi1* plants, we hypothesized that salicylic acid (SA)-mediated defenses may play a prominent role in *coi1* resistance. Here we show that *coi1* resistance to *P. syringae* is correlated with rapid and strong induction of defenses associated with the SA-pathway. To further explore the role of SA-mediated defenses in *coi1* resistance, we generated *coi1 npr1* double mutants and a *coi1* line carrying the NahG transgene. Both of these *coi1* "double mutant" lines allow high levels of bacterial growth, but appear resistant to *P. syringae* at the level of symptom production. These results suggest that rapid induction of the SA-mediated defense pathway is key in the suppression of bacterial growth in *coi1* tissues, but that symptom production is dependent on the wild type COI1 allele.

157 Isolation and characterization of *Arabidopsis thaliana* mutants that have reduced susceptibility to *Pseudomonas syringae* pv. tomato strain DC3000

Paula Lee, Lisa Dinolfo, Anne Plovanch-Jones, Ruth Nantais, Vusumuzi Ndlovu, Sheng Yang He

Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48823

The molecular basis of plant susceptibility to pathogen infection remains largely elusive. Currently, much research has focused on defense and gene-for-gene based resistance. Our research focuses on plant susceptibility to the bacterial pathogen *Pseudomonas syringae* pv. tomato strain DC3000 (Pst DC3000). We are screening for *Arabidopsis thaliana* mutants that have reduced disease symptom development. From a screen of about 10,000 ethylmethane sulfonate-mutagenized *A. thaliana* ecotype Columbia g11 plants, we found 3 *edr* (enhanced disease resistant) mutants: *edr103*, *edr207*, and *edr209*. *edr103* is defective specifically in chlorosis development. However, Pst DC3000 multiplication and necrosis development are normal in this mutant. *edr207* and *edr209* are putative *cpr* (constitutive expressor of pathogenesis-related genes)-type mutants that have reduced bacterial multiplication as well as constitutively high levels of PR1 gene expression. We are also screening for decreased susceptibility mutants in the *A. thaliana npr1-2* (nonexpressor of pathogenesis-related genes) background. *npr1* plants are defective in salicylic acid-induced resistance, are generally more susceptible to attack by Pst DC3000, and show greater disease symptom development. By using this genetic background in a screen, we will identify mutants that are specifically affected in susceptibility to Pst DC3000, not *cpr*-type and lesion mimic-type mutants. Three putative mutants have been recovered from a screen of 25,000 M2 plants.

158 Identification of genes involved in rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis*

Karen M. Léon-Kloosterziel, Joost J.B. Keurentjes, Bas W.M. Verhagen, Corné M.J. Pieterse, Leendert C. van Loon

Section of Plant Pathology, Utrecht University, P.O. box 80084, 3508 TB Utrecht, The Netherlands

Different forms of induced resistance have been identified in plants. Systemic acquired resistance (SAR) is induced upon infection with a necrotizing pathogen and is associated with PR gene expression and salicylic acid (SA) accumulation. Several non-pathogenic *Pseudomonas* bacteria can induce systemic resistance as well, without causing any symptoms. In this induced systemic resistance (ISR) response, SA is not involved. Instead, ISR requires responsiveness to both jasmonate and ethylene. PR proteins do not accumulate during ISR. To identify genes that are expressed upon induction of ISR, we use a collection of *Arabidopsis thaliana* gene trap and enhancer trap lines containing the GUS reporter gene. We screened for GUS-expression upon root colonization by the non-pathogenic rhizobacterium *Pseudomonas fluorescens* strain WCS 417r, which is effective in inducing ISR in *Arabidopsis*. One enhancer trap line has been found that showed GUS expression in the roots after treatment with several strains of ISR-inducing *Pseudomonas* bacteria. This line also showed GUS expression in response to ACC, but not to jasmonate or SA. The inducible gene in this line is a thaumatin-like gene that shares homology to PR5. Expression studies are in progress.

159 Uncoupling salicylic acid-dependent cell death and defense-related response from disease resistance in the *Arabidopsis* mutant *acd5*

Hua L. Liang, F. Paul Silverman*, and Jean T. Greenberg

Dept of Molecular Genetics & Cell Biology, University of Chicago, *Valent Biosciences Corporation, Longgrove, IL

Salicylic acid (SA) is required as a signal molecule to induce systemic acquired resistance in plants. It is required for hypersensitive cell death induction in plant defense responses to many pathogens. We isolated and characterized a mutant called accelerated cell death 5 (*acd5*) in *Arabidopsis thaliana* that shows spontaneous cell death that resembles a susceptible bacterial infection primarily in younger leaves and stems late in development. We mapped *acd5* to chromosome 5, in the 20 cM region between *LFY* and *DFR* markers. SA, ethylene, PR1, BGL2 and camalexin levels were elevated in *acd5* plants showing lesions. However, *acd5* was not more resistant to *P. syringae* or *X. campestris* than wild type, indicating that SA, cell death and defense-related responses are uncoupled from resistance. To determine if SA is required for *acd5* phenotypes, *acd5-nahG* plants were produced. We found that *nahG* dominantly suppressed the cell death of *acd5* plants. Application of BTH (a SA agonist) to *acd5-nahG* plants reversed the suppression. The Nonexpressor of PR1 (*NPR1*) gene is required for some aspects of SA signaling. In *acd5npr1* double mutants, *acd5* phenotypes were highly attenuated. Treatment of BTH or SA on these plants didn't reverse the suppression, indicating that *NPR1* is essential for mediating SA-induced cell death, while SA is necessary and sufficient to induce cell death. To investigate ethylene's role in the cell death phenotype of *acd5* , we crossed *acd5* with the ethylene insensitive *ein2* mutant in which ethylene signaling is blocked. *Ein2* partially suppressed the *acd5* phenotypes. We propose that *acd5* plants mimic susceptible pathogen infections and that SA and ethylene can play a role in susceptible cell death regulation distinct from their roles in influencing pathogen resistance.

160 A pectin lyase homologue from *Pseudomonas syringae* is induced during the infection of *Arabidopsis thaliana*

Melisa Lim and Barbara Kunkel

Washington University, St Louis, MO

The mechanisms that bacteria use to become pathogenic on plants are not clearly understood. Once inside the plant, pathogens need to acquire nutrients and cope with host defense responses. To achieve this, plant pathogens turn on the expression of numerous genes. Therefore to investigate the processes important for pathogenicity and for virulence, we carried out a screen for genes preferentially expressed by the biotrophic bacteria *Pseudomonas syringae* tomato (*Pst*) DC3000 during its infection of *Arabidopsis thaliana* (see poster on IVET screen, V. Joardar et al.). One gene identified during this screen shows highest homology to a pectin lyase (*pnl*) gene from *Bacillus subtilis*. As expected, the expression of the *pnl* homologue is low on rich medium and increases in minimal medium and in planta. Analysis of sequences upstream of the putative start codon revealed the presence of a possible Hrp-box characteristic of genes whose expression are dependent on the Hrp L sigma factor. The latter regulates the expression of other genes that have been shown to be required for pathogenicity. Furthermore a fusion of the *pnl* 5' sequences to the *uidA* gene (*GUS*) exhibited higher levels of expression in *E.coli* in the presence of a plasmid bearing *hrp L*. A strain of *Pst* with a disruption of the *pnl* gene was able to cause disease on *Arabidopsis* ecotype Col-O and on tomato. In addition, the mutant was capable of mediating a HR on tobacco and also on

wild-type *Arabidopsis* when the bacteria expressed several different *avr* genes. These results indicate that despite its high levels of expression in planta, *pnl* is not absolutely required for virulence of *Pst* on the ecotypes of *Arabidopsis* and tomato tested. To uncover a subtler role for the *pnl* gene, the *pnl* mutant was transformed with a plasmid bearing the gene *avrRpt2*. The latter has been shown to promote disease caused by *Pst* on *Arabidopsis* (see poster by Z.Chen et al.). Surprisingly, the *pnl* mutant expressing *avrRpt2* was not more virulent on *Arabidopsis* than the mutant without *avrRpt2*, indicating that the *pnl* gene is important for the expression of the virulence activity of *avrRpt2* by *Pst* DC3000. The reason for this is being investigated.

161 Induction of *A. thaliana* WRKY proteins upon challenge with *Peronospora*

parasitica

Bernadette Lippok, Koen Dekker, Klaus Hahlbrock, and Imre Somssich.

Max Planck Institute for Plant Breeding Research, Carl-von-Linne-Weg 10, D-50829

Koeln, Germany

WRKY proteins comprise a family of plant-specific zinc-finger-type factors implicated in the regulation of genes associated with pathogen defense. In parsley, individual members of this family have been shown to be induced upon elicitor treatment (1). In *Arabidopsis*, over 60 members are presently annotated in the databases (2). Within our functional genomics project, called ZIGIA, we are following a genetic approach to identify gene-function-relationships of WRKY genes. As a first approach we are identifying WRKY genes which are expressed during the defense reaction of *Arabidopsis* when infected with avirulent strains of *Peronospora parasitica*. Next, knock-out plants for the identified WRKY genes are selected by PCR-screening of our collection of 11000 (ca. 65 000 independent insertions) *En/Spm* carrying *Col-0* lines. These plants are screened for phenotypes after infection with *Peronospora parasitica*. Moreover, Northern analyses are carried out and promoter:GUS fusion carrying plants are made to better understand where and under which conditions these WRKY genes are expressed.

(1) Eulgem, T et al., *EMBO J.* 1999, 18(17):4689-99.

(2) Eulgem, T et al., *TIPS* 2000, 5: 199-206.

162 A Novel Role for Salicylic Acid in Cell Fate Determination in *Arabidopsis*

Leaves

Hua Lu, Hélène Vanacker, Jean T. Greenberg

Department of Molecular Genetics and Cell Biology, University of Chicago, 1103 East

57th Street, Chicago, IL 60637

Salicylic acid (SA) is an important defense molecule in higher plants. It can act on its own or with other signal(s) to induce resistance to a broad spectrum of pathogens, to amplify other defense signals, and to cause cell death. For the first time, we show here that SA induced cell death is coupled with cell growth in an *Arabidopsis* mutant. *acd6-1* is a dominant gain-of-function mutant, showing spontaneously cell death and cell enlargement in leaf mesophyll tissue. Depletion of SA by crossing *acd6-1* with a *nahG* plant, a transgenic plant that overexpresses an enzyme inactivating SA, suppresses the *acd6-1* phenotype. However, reactivating the SA signaling pathway using the synthetic SA analog benzo(1,2,3)thiadiazole-7-carbothioic acid (BTH) hyperactivates the

acd6-1 phenotype. acd6-1nahG plants were thus treated with 10 μ M BTH and leaves were collected at different time points and embedded in histocryl resin. From the crossed sections of leaf tissues, we found that dead cell fragments and enlarged cells were adjacent to each other within the first 24 hr treatment. Cell death and cell enlargement continued at day 2, 3, and 4, creating some airspace and giant cells. By 8th day, a few enlarged cells were surrounded with many smaller cells. The cell number between two secondary veins in the abnormal growth regions was higher than that in the control plants, suggesting cell division was activated. In addition, endoreduplication was induced in the enlarged cells based on quantitation of nuclear DNA content. Since SA alone does not affect cell fate in wild type plants, we propose that SA may interact with at least one additional yet unidentified signaling pathway in the mutant background to produce novel cellular responses. In addition, we also found that *Pseudomonas syringae* pathogen attack can activate cell growth and cell death. We are currently investigating the role of SA in this activation.

163 Genetic analysis of *Arabidopsis* nonhost resistance to *Pseudomonas syringae* pv *phaseolicola*

Ming Lu, Xiaoyan Tang, and Jian-Min Zhou

Kansas State University

Nonhost resistance is fundamental to plant-microbe interactions. Despite many breakthroughs in gene-for-gene resistance studies, little is known about why a pathogen fully pathogenic on its host plant species is non-pathogenic on nonhost plant species. We propose that general defense plays an important role in limiting the growth of bacterial pathogens in a nonhost plant. The suppression of general host defense is one of the prerequisites for the evolution of virulent pathogens. We are investigating the interaction between *Arabidopsis* and its nonhost pathogen *Pseudomonas syringae* pv *phaseolicola* NPS3121. Our results demonstrate that HR-independent defense mechanisms play an important role in limiting *P. s. phaseolicola* growth in *Arabidopsis*. In addition, we have isolated 10 novel *Arabidopsis* mutants with reduced nonhost (nho) resistance to *P. s. phaseolicola*. Three mutants, nho1101, nho1409, and nho1531 have been characterized further. nho1101 and nho1409 were significantly compromised in disease resistance conferred by the R genes, RPS2, RPM1, and RPS4, whereas nho1531 had a slight reduction in RPM1-mediated resistance. Complementation tests indicate that the mutations define three complementation groups. nho1409 and nho1531 both carry a single recessive mutations. nho1409 has been mapped to the bottom of chromosome I, a region containing no known disease resistance mutations. The results indicate that nonhost resistance is amenable to genetic analysis in *Arabidopsis*. The characterization of nho mutants and the future isolation of NHO genes will shed light on general defense mechanisms in plants and their relationship with gene-for-gene resistance mechanisms.

164 Accelerated Cell Death 2: a novel gene that suppresses cell death

Mach, J.M. and Greenberg, J.T.

University of Chicago; Chicago, IL

When a plant recognizes a pathogen, it initiates a suite of defenses, including production of reactive oxygen, transcription of defense genes, and activation of cell death. In plants mutant for the accelerated cell death gene ACD2, these defenses are activated in the

absence of pathogen. We have cloned and characterized the ACD2 gene and find that it encodes a novel protein that may localize to the chloroplast or mitochondrion. The endogenous protein migrates at a smaller molecular weight than in vitro translated protein by SDS-PAGE, indicating that it is processed, possibly during import. Deletion of the putative transit peptide abolishes ACD2 function, indicating that organellar import may be required for function. To determine whether ACD2 protein functions cell autonomously, we have made constructs to produce mosaic plants. Details on ACD2 localization and cell autonomy will be presented. We have also found that ACD2 protein diminishes in abundance early in senescence, indicating that it may function in multiple cell death processes. We have constructed plants that produce an excess of ACD2 protein by expressing the cDNA under the control of the CaMV 35S promoter; phenotypic analysis of these plants will be presented.

165 *Pseudomonas syringae* type III effector proteins AvrRpm1 and AvrB induce distinct responses on resistant and susceptible *Arabidopsis* hosts

David Mackey¹, Zachary L. Nimchuk¹, Susanne Kjemtrup¹, and Jeffery L. Dangl^{1,2}
¹Department of Biology and ²Curriculum in Genetics, University of North Carolina at Chapel Hill, 108 Coker Hall, Chapel Hill, NC, USA 27599-3280

Type III secretion systems are conserved virulence systems which allow phytopathogenic bacteria to inject effector proteins into host plant cells. Avr proteins constitute a diverse class of effectors which are active in the host cell. Many Avr proteins have dual roles as virulence determinates on susceptible hosts and recognition determinants on resistant hosts. It is unclear what features of Avr proteins are required to perform these dual functions. Using the *Pseudomonas syringae*-*Arabidopsis* model system we have begun to explore the biology of AvrB and AvrRpm1 function on susceptible and resistant hosts. We have identified targeting signals on AvrB and AvrRpm1 which are required for association with the host plasma membrane. We will present additional data on structural requirements for AvrRpm1 and AvrB functions on resistant and susceptible hosts.

166 Expression profiling of compatible interaction between *Arabidopsis* and biotrophic fungal pathogens

R. Mahalingam, R. Raina and N. V. Fedoroff

Life Sciences Consortium, The biotechnology Institute, Pennsylvania State University, University Park, PA 16802, USA

Biotrophic plant pathogens such as *Peronospora parasitica*, invade living cells and manipulate the host metabolism to favor their growth and reproduction, without killing the host. These biotrophs possess the finesse to evade recognition by the host defense machinery. An *Arabidopsis* (Columbia ecotype) cDNA library enriched for genes induced in response to infection by the virulent *P. parasitica* strain Ahco2 was constructed using PCR-suppression subtractive hybridization (SSH). About 750 clones from the forward subtraction (mRNA from fungus-infected plants used as tester, water-treated control plants as driver) and 250 clones from the reverse subtraction (mRNA from fungus infected plants used as driver) were subjected to differential screening using the subtracted and unsubtracted cDNAs. Based on this screening, 236 unique cDNAs from the forward library and 18 cDNAs from the reverse library were selected and sequenced. More than 40% (96 clones) of these clones are not represented in the *Arabidopsis* EST

database. These SSH clones, along with clones from three other stress libraries (ozone, salicylic acid and avirulent bacteria) and a set of microarray controls were spotted on silanized glass slides using a cDNA microarray robot. Plants infected with fungus (*P. parasitica* strain Ahco2 or *Erysiphe cichoracearum*) and corresponding controls were harvested at 24-hour interval starting one day after inoculation up to seven days. Total RNA isolated from plants harvested at each of these time points was reverse transcribed, and the cDNAs were coupled to fluorescent dyes and used as probes on the DNA microarray. Scanned arrays were analyzed using the Imogene software (Biodiscovery). Host genes induced during the intricate interplay between the host and biotrophic pathogens provide valuable information about plant components in a compatible plant-pathogen interaction and improve our understanding of signaling during disease development. Expression profile along with sequence information of the genes provides a strong rationale for selecting candidate genes for pursuing our practical goal of improving stress tolerance in plants.

167 Developmental Regulation of Resistance to Downy Mildew in *Arabidopsis*
John M. McDowell*, Scott Williams, Thomas Eulgem, and Jeffery L. Dangl
Department of Biology, University of North Carolina, Chapel Hill, NC *Current Address:
Fralin Biotechnology Center, Virginia Tech, Blacksburg, VA

Host resistance to agronomically significant pathogens can be developmentally regulated in certain instances. This differential expression can decrease the efficiency of resistance, and it would be of interest to determine the underlying molecular cause(s). We have observed that race-specific resistance to *P. parasitica* Emco5 is differentially regulated during development of Col-0. 7 day old seedlings support profuse mycelial growth and asexual spore formation in the cotyledons. Emco5 growth is dramatically (but not completely) restricted in the first set of true leaves, and subsequent leaves exhibit progressively increased resistance. This apparent "adult resistance" is suppressed by expression of the NahG transgene in Col-0 and by mutant alleles of the NDR1, PBS2, PBS3, NPR1, and PAD4 loci. In contrast to Col-0, Ws-0 supports profuse growth of Emco5 at all stages of development. Analysis of Col-0 X Ws-0 F1 hybrids and segregating F2 populations indicate that susceptibility in Ws is dominant to resistance in Col-0. This observation implies that Ws-0 expresses a dominant suppressor of developmentally regulated resistance in Col-0. This dominance is of interest since alleles for susceptibility are typically recessive to alleles specifying resistance. We are currently determining the number and map position of segregating loci in a Col-0 X Ws-0 F2 population as a prelude to positional cloning.

168 PBS2, a putative signaling intermediate in the *Arabidopsis thaliana* disease resistance pathway

P.M. Merritt, R.F. Warren, and R.W. Innes

Department of Biology, Indiana University, Bloomington, Indiana 47405, USA

The gene-for-gene hypothesis is a widely accepted model for plant pathogen interactions. According to this model, plants are able to mount a resistance response if they express a resistance gene (R gene) that corresponds to a pathogen expressed avirulence gene (avr gene). Previous work in our lab has led to the identification and isolation of the R gene RPS5 in *Arabidopsis thaliana*. The RPS5 mutant screen identified *Arabidopsis* plants that

had lost resistance to the gram-negative bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000 (Pst DC3000) expressing the avr gene avrPphB. This screen also identified three putative members of the disease resistance signaling pathway. These signaling genes are called PBS1, PBS2 and PBS3 for avrPphB susceptible. In addition to avrPphB susceptibility, the pbs2 mutant is also susceptible to Pst DC3000 expressing avrB, avrRpm1 and avrRpt2 while resistance to Pst DC3000 expressing avrRps4 is maintained. Mutation of PBS2 also causes a loss of resistance to several strains of the fungal pathogen *Peronospora parasitica*. This phenotype suggests that PBS2 functions in a pathway that receives input from R genes of the non-TIR class of NBS-LRR genes. A PCR based mapping strategy has been employed in order to isolate the PBS2 gene. Initial mapping data located PBS2 to the north end of chromosome I. Analysis of 1287 F2 mapping lines (pbs2 x WS) has identified five individual F2 plants with recombination events between the markers nga63 and NCC1 giving a genetic distance of 0.24cM. Further analysis has limited the physical region containing PBS2 to approximately 350kb. Three overlapping and fully sequenced BAC clones span this region. The current cloning strategy includes the sequencing of candidate genes in pbs2 mutants and the construction of cosmid libraries for transformation of mutant plants.

169 Mutational analysis of EDS1 dependent disease resistance signaling

Matthew Metz, Walter Gassmann, & Brian Staskawicz

Department of Plant and Microbial Biology, 111 Koshland Hall, University of California, Berkeley, CA 94720

One mechanism of disease resistance in plants is mediated by resistance (R) genes in the plant that specify recognition of pathogens that express a corresponding avirulence (avr) gene. In *Arabidopsis* multiple signaling pathways exist that are utilized by mutually exclusive sets of R genes for resistance function. The EDS1 dependent pathway is used by RPS4 in specifying resistance to bacteria expressing avrRps4. In a previous screen for mutants of RPS4 one mutant eds1-4 was obtained from 3,000 Fast Neutron M2 plants. We are screening EMS mutagenized seed for mutants with compromised resistance to *Pseudomonas syringae* DC3000 expressing avrRps4. We anticipate the likelihood of finding mutations in RPS4, EDS1 and other genes that are important for the operation of this particular signaling pathway. We have screened 15,000 M2 seed and found four mutants that breed true in the M3 generation. Complementation analysis and genetic mapping of these mutants are under way.

170 The GI and FCA loci participate in disease response during cauliflower mosaic virus infection of *Arabidopsis*

Joel J. Milner¹, Simon N. Covey², George Coupland² & Edi Cecchini¹

¹Glasgow University, Glasgow, Scotland; ²John Innes Centre, Norwich, England

Our objective is to identify and characterize signalling pathways in disease response to compatible virus infections. Under short day regimes, when plants have an extended vegetative life span, systemic infection by cauliflower mosaic virus (CaMV) induces severe symptoms in *Arabidopsis*. Symptoms include vein-chlorosis in expanded leaves, and severe distortion, stunting and chlorosis in developing leaves. In long days, which promote early flowering, infected plants develop only mild symptoms. We have used a reverse genetic approach to investigate the involvement of two loci that affect flowering,

FCA and GI, in disease response. These loci lie on different signalling pathways; *fca* mutants are late flowering in short and long days, whereas *gi* mutants are late flowering in long days only. In long days, CaMV infected *fca-1* and moderate *gi* alleles, *gi-2* & *gi-3* developed severe vein clearing in expanded leaves and severe chlorosis, distortion and stunting of the developing leaves. However, a T-DNA knockout, *gi-11*, was almost asymptomatic. Despite the differences in symptom severity and character, we could detect no differences in the levels of replicating virus accumulating in either expanded or developing leaves between any of the *gi* alleles and wild-type controls. In short days, infected *fca-1* developed similar symptoms to wild-type. However, *gi-11* was almost asymptomatic, and in moderate alleles, *gi-1*, *gi-2* & *gi-3*, expanded leaves developed vein clearing but developing leaves showed no chlorosis and greatly reduced distortion. Under short- as under long days, levels of replicating virus in all the *gi* alleles were indistinguishable from wild-type controls. These mutations, the first of this type to be identified, alter symptom response but do not appear to affect virus accumulation. Their existence provides compelling genetic evidence for specific disease-response signalling pathways that can be de-coupled from virus accumulation. Transition to flowering is an important factor modulating symptom-response. However, symptoms in *gi* mutants are also affected in short days indicating an additional role for GI in controlling symptom expression.

171 Map-based cloning of TTR1: a TIR-NBS-LRR gene that induces hypersensitive disease in Tobacco ring spot virus-infected Arabidopsis plants

Jae Sun Moon, Hua Jin, Jian Ming Lee, Andrew F. Bent, Glen L. Hartman, and Leslie L. Domier

University of Illinois

When Columbia Arabidopsis plants (Col-0) are infected by Tobacco ring spot virus (TRSV), they display no obvious symptoms. In contrast, Eastland Arabidopsis plants become systemically necrotic and die within two weeks of inoculation. Previous genetic studies revealed that symptom expression is controlled by a single semidominant gene, TTR1. The gene was mapped to an interval of approximately 80-kb on chromosome V. Using the sequence for this region, DNA fragments representing predicted genes and flanking regions were analyzed in reciprocal transformation-complementation tests. In preliminary studies, two Col-0 lines transformed with a 6.7-kb fragment of Eastland genomic DNA developed systemic necrosis upon TRSV infection. The sequence of this fragment indicated that it contained a 4.9-kb gene, with six introns that can encode a 155-kDa protein. The predicted amino acid sequence of the protein showed significant similarity to the sequences of TIR-NBS-LRR resistance gene products, including those of RPS4, L6, and N, which are responsible for hypersensitive resistance to bacterial, fungal, and viral pathogens, respectively. These data suggest that Col-0 tolerates TRSV infection because it fails to produce a hypersensitive resistance response to the pathogen, and further suggest that disease sensitivity in Eastland may be due to the necrotic response conditioned by a TIR-NBS-LRR resistance gene.

172 SNI1, an Elusive Element in Systemic Acquired Resistance

Rebecca A. Mosher, Xin Li, and Xinnian Dong

Duke University

Systemic Acquired Resistance (SAR) is characterized by an increase in salicylic acid (SA) levels which causes upregulation of pathogen-related (PR) genes and subsequent resistance to a broad range of pathogens. NPR1 (non-expressor of PR genes) is a critical positive regulator of SAR known to act downstream of the SA signal and upstream of PR gene induction. *sni1* (suppressor of *npr1-1*, inducible) restores inducible resistance to *npr1* mutants. In an NPR1 background, the *sni1-1* allele (presumed null) seems to confer resistance at levels of inducer insufficient for WT induction. The SNI1 sequence has no obvious homology or known domains, though orthologs have been found in other plant species. SNI1 is hypothesized to be a repressor of PR gene expression which is removed by NPR1 upon induction of SAR. Further characterization of *sni1-1* and functional characteristics of SNI1 will be discussed.

173 R-Protein degradation induced by Avr-Proteins

Jaesung Nam¹, David Mackey², Claire H. Taylor², and Jeffrey L. Dangl^{2,3}

¹X ²Department of Biology and ³Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC, 27599-3280

RPM1 is an R-gene that specifies recognition of the bacterial avirulence gene *avrRpm1*. Bacteria which deliver AvrRpm1-protein via type III secretion induce the hypersensitive response (HR) on RPM1 plants. A derivative of RPM1 with a carboxy-terminal tag, expressed under control of its native promoter, complements *rpm1* mutations and can be detected in planta. This derivative is peripherally associated with the plasma membrane. Upon induction of HR by AvrRpm1, RPM1 is degraded prior to the onset of macroscopic HR (Boyes, Nam, and Dangl. 1998. PNAS). When HR is triggered by RPS2 or RPS4, RPM1 is similarly degraded prior to HR. The degradation of RPM1 is being characterized in various mutants in the disease resistance pathways. In *ndr1* mutants, degradation of RPM1 is induced by RPS2 in the absence of macroscopic HR. This indicates that the signal which induces R-gene degradation may diverge prior to the induction of cell death. Proteasome inhibitors block degradation of RPM1 and lead to a more severe HR. Thus, R-gene degradation may attenuate the severity of the HR.

174 TAO mutants of Arabidopsis are defective in susceptible responses to the *Pseudomonas syringae* type III effector AvrB

Zachary L. Nimchuk¹, Christina L. Helms¹, and Jeffery L. Dangl^{1,2}

¹Department of Biology, and ² Curriculum in Genetics, University of North Carolina at Chapel Hill. 108 Coker Hall, Chapel Hill, NC, USA 27599-3280

Invading bacteria use several strategies to successfully establish infection on plant hosts. One evolutionarily conserved strategy is to deploy effector proteins into the host cell via the type III secretion system. Genetic analysis has identified Avr proteins as effectors of the type III secretion system in phytopathogenic bacteria. While many of these Avr proteins are known to contribute to virulence on susceptible hosts, the mechanism by which they do so is unknown. The delivery of Avr proteins to the interior of host cells places them in position to interact with and influence host signalling components. We would like to identify components and pathways which are targeted by Avr proteins during infection on susceptible hosts. We have taken a genetic approach to identify targets of the *Pseudomonas syringae* type III effector AvrB on susceptible Arabidopsis ecotypes. AvrB induces a chlorotic response when expressed in susceptible ecotypes.

This response requires association of AvrB with the host plasma membrane via N-terminal myristoylation. Using a conditional expression system, we have identified Arabidopsis mutants which fail to exhibit AvrB-induced chlorosis. These Target of AvrB Operation (TAO) mutants may help illuminate how bacteria cause disease in susceptible hosts. Data will be presented on the characterisation of the TAO mutants and genetic analysis of AvrB-induced chlorosis.

This work is supported by DOE grant DE-FG05-95ER20187 to JLD.

175 Cloning and characterization of mildew-induced lesion mutants

Marc Nishimura

Carnegie Institution

The mil (mildew-induced lesions) mutants were identified in a screen looking for increased resistance to the fungal pathogen powdery mildew (*Erysiphe cichoracearum*). These resistant mutants abnormally form necrotic lesions ~7 days after inoculation. I am currently placing these mutants into complementation groups and characterizing their lesion and resistance phenotypes. The lesion phenotype of the mutants will be examined using a broad range of biotic and abiotic stresses. Mutants that form lesions specifically to pathogens or a subset of pathogens will be pursued for further characterization and cloning. By cloning and characterizing mil mutants I hope to discover novel components of the signal transduction pathways leading to defense responses.

176 Characterization of pbs3: a mutant with enhanced susceptibility to *Pseudomonas syringae*

Kan Nobuta(1), Randall F. Warren(1), Klaus Maleck(2), and Roger W. Innes(1)

(1)Indiana University, Department of Biology, Bloomington, IN (2)Novartis, Research Triangle Park, NC

The RPS5 gene of Arabidopsis mediates resistance to strains of the bacterial pathogen *Pseudomonas syringae* pv tomato (Pst) that carry the avirulence gene *avrPphB*. To identify genes that are required for RPS5-mediated resistance, we inoculated mutagenized Col-0 plants with Pst strain DC3000 (*avrPphB*), and those with disease symptoms were analyzed. Three loci, designated PBS1, PBS2, and PBS3 were identified (Genetics, 152:401-412). The *pbs3* mutant shows enhanced susceptibility not only to PstDC3000 (*avrPphB*), but also to DC3000 strains that carry *avrRpt2*, *avrB*, or *avrRps4*. In all cases, however, the level of bacterial growth was intermediate between a fully resistant and a fully susceptible interaction. In addition, *pbs3* mutant plants displayed enhanced susceptibility to a normally virulent DC3000 strain containing no added avirulence genes. These data are similar to those obtained with transgenic Arabidopsis plants that express NahG, a bacterial gene that encodes salicylate hydroxylase. Therefore, we analyzed SA levels in *pbs3* plants inoculated with PstDC3000. SA levels in the *pbs3* mutant were significantly lower than in Col-0 wild-type plants. We also looked at PR1 gene expression in the *pbs3* mutant. Plants were vacuum-infiltrated with PstDC3000 (*avrRpt2*), and tissue samples were collected 12 and 24 hours later. The *pbs3* mutant showed a very low level of PR1 gene expression, compared to that of Col-0 wild-type. We also looked at the defense response of *pbs3* mutants to *Erysiphe cichoracearum*, which is a causal agent of powdery mildew. Surprisingly, the *pbs3* mutant showed an

enhanced resistance phenotype to *E. cichoracearum*. We are now determining whether the susceptibility to PstDC3000 strains and the resistance to *E. cichoracearum* co-segregate in the *pbs3* mutant.

177 Gene Expression During Resistance and Tolerance to the Black Rot Pathogen
Picanso, E., Wilson, I. W., Nishimura, M., Somerville, S. C., and Buell, C. R.
The Institute for Genomic Research, 9712 Medical Center Dr, Rockville MD, 20850 and
The Carnegie Institution of Washington, Department of Plant Biology, Stanford CA
94305

Both resistance and tolerance to *Xanthomonas campestris* pv *campestris* have been demonstrated in *Arabidopsis thaliana*. Major genes conferring tolerance (RXC1) and resistance (RXC2, RXC3, and RXC4) have been identified in differential accessions of *Arabidopsis*. Additional loci involved in resistance and tolerance have been identified using a mutational approach and include the developmental loci *hookless1* (*hls1*), constitutive photomorphogenic 2 (*cop2*), ethylene insensitive 2 (*ein2*), and *ein6*. To further understand how resistance and tolerance are manifested at the molecular level, we measured gene expression levels in resistant and tolerant tissues of over 500 genes implicated in disease resistance using cDNA microarrays. The three wild-type accessions, Columbia, Landsberg erecta, and Pr-0, and the *hls1* mutant were examined using microarrays. The expression levels of genes that were differentially regulated in these studies were further examined in an extended time course using northern analyses. From these studies, we were able to identify expression patterns associated with resistance and tolerance.

178 Characterization of a gain-of-function mutant of *Arabidopsis*, which spontaneously develops lesions mimicking disease
Rachel Pilloff, Sendil Devadas, and Ramesh Raina
The Pennsylvania State University, University Park, PA 16802 USA

A salient feature common to both compatible and incompatible plant-pathogen interactions is host cell death. Here we describe the characterization of a novel gain-of-function *Arabidopsis* mutant, *dll1* (disease-like lesions), which spontaneously develops disease-like lesions. These water-soaked lesions surrounded by regions of chlorosis appear on four-week old plants and mimic bacterial speck disease. Following lesion formation, *dll1* plants constitutively express biochemical (autofluorescent material, callose, hydrogen peroxide) and molecular (PR-1, PR-2, PR-5, GST-1) markers associated with pathogen infection. Despite the constitutive expression of these defense-related markers, *dll1* is not able to repress the growth of virulent bacterial or fungal pathogens. However, *dll1* triggers normal HR in response to avirulent bacterial pathogens, thus indicating that the gene-for-gene mediated resistance response is functional in these plants. Furthermore, *dll1* mutant supports the growth of *hrp-* mutant strains of *P. syringae*, which are unable to grow on the wild type parent Col-0, suggesting that *dll1* phenotype looks like disease and causes the same cellular processes that are required by pathogens for their growth. These results suggest that *dll1* constitutively expresses many of the features associated with plant response to virulent pathogens during compatible interactions. This suggests that host cell death during compatible interactions, at least in part, is genetically controlled by the plant, and that DLL1

positively regulates this process. Further characterization of the *dll1* mutant and the cloning of the corresponding gene should help increase our knowledge of the signaling pathways involved in cell death during disease.

179 PLANTS AS MODEL HOSTS TO STUDY PSEUDOMONAS AERUGINOSA PATHOGENESIS

Laurence G. Rahme

Department of Surgery, Harvard Medical School and Department of Surgery, Massachusetts General Hospital and Shriners' Burns Institute, Boston 02114

Our goal is to gain insight into the molecular interactions that underlie the pathogenicity of *Pseudomonas aeruginosa*, a gram-negative human opportunistic pathogen that also infects plants and insects. A clinical isolate of *P. aeruginosa* strain PA14 elicits soft-rot disease symptoms and proliferates in *Arabidopsis* leaves. Studies of the structure and distribution of *P. aeruginosa* strain PA14 in *Arabidopsis* leaves using Nomarski technique and confocal microscopy, showed that *P. aeruginosa* penetrated the plant mesophyll cells within 24 hours. Infected leaves revealed the presence of *P. aeruginosa* in the intercellular spaces of the leaves as well as bacterial movement along the veins and bacterial proliferation in vessel parenchyma and companion cells. No bacteria are detected in the xylem. Fast propagation of *P. aeruginosa* in the vessel parenchyma is the prelude to systemic infection and maceration of the entire plant. Using *Arabidopsis thaliana*, we have shown that *P. aeruginosa* can cause disease in both plants and animals using a shared subset of virulence factors. This finding leads us to the approach of using plants as genetically tractable hosts for the direct screening of virulence factors relevant to pathogenesis in mammals. Screening of 3,000 transposon-generated mutants of *P. aeruginosa* strain PA14 for reduced virulence in plants allowed us to identify previously unknown *P. aeruginosa* pathogenicity-related genes and uncover known mammalian pathogenicity mechanisms which are relevant in plant pathogenesis. The majority of the mutants thus identified in plants displayed reduce virulence in a burned mouse model. Most of the virulence-associated genes of *P. aeruginosa* thus identified have been cloned. They encode proteins involved in transcriptional and post-translational control, efflux systems, and biosynthetic enzymes involved in phenazine production, virulence effectors as well as proteins of unknown function. We are now undertaking molecular and biochemical studies to characterize these genes and elucidate their role during infection.

180 The Relationship of EDS1, PAD4 and SA to LSD1 in Arabidopsis Disease Resistance

C. Rusterucci *1, D. H Aviv *2, B. J. Feys 1, R. A. Dietrich 2, J. L. Dangl 2, and J. E. Parker 1

1 Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, U.K. 2 Department of Biology and Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC 27599, U.S.A.

The EDS1 (Enhanced Disease Susceptibility) and PAD4(Phytoalexin Deficiency) genes are major signalling components in *Arabidopsis* race-specific resistance to the bacterial pathogen *Pseudomonas syringae* and to the oomycete, *Peronospora parasitica*. They also contribute to the restriction of growth of virulent pathogens. Both gene products have homology to eukaryotic lipases and act upstream of salicylic acid (SA)-mediated

defences. They also interact in a yeast-two hybrid assay. We have examined the role of EDS1 and PAD4 with respect to the generation of reactive oxygen intermediates (ROI), an early response, and to the elaboration of the plant hypersensitive response (HR), a later reaction, in plants challenged with pathogens. We find that whilst *eds1* suppresses both ROI accumulation and the HR, *pad4* does not affect ROI generation and allows a weak HR to occur. We have also assessed the relationship between these and several other Arabidopsis defence signalling mutations (*NahG* plants depleted in SA, *npr1*, and *ndr1*) with the *lsd1* mutation that gives rise to runaway plant cell death upon pathogen inoculation or treatment with SA. We present results that show different levels of interaction between these signalling components. Significantly, both *eds1* and *pad4* suppress *lsd1*-induced runaway cell death but depletion of SA and the *ndr1* mutation do not in the pathogen-induced responses. We use these data to build a model for the function of these components in plant disease resistance.

* co-first authors

181 MIL1, a novel gene required for powdery mildew susceptibility in Arabidopsis
Céline Schiff, John Vogel and Shauna Somerville
Carnegie Institution of Washington

To address the question of the roles of cell death in resistance to pathogens, a screen was undertaken to isolate Arabidopsis mutants with an increased resistance associated with cell death when infected by the powdery mildew *E. cichoracearum*. The characterization and cloning of one recessive mutant, *mil1* (mildew induced lesions) are presented here. To characterize the lesion phenotype, both fungal growth and cell death were monitored at various times after inoculation. Typically, the fungus grew at near wild type levels early in the infection. However, at later time points when lesions began to form, fungal development was severely altered (up to 83% reduction in conidiation). The timing of lesion formation varied with the density of inoculation, 4 days post inoculation (dpi) at 200 spores/mm² vs. 6 dpi at 20 spores/mm², and coincided with the stage of development where the fungal growth was reduced. Importantly, *mil1* failed to form lesions in response to all abiotic stresses tested (wounding, low and high temperatures, light quality and length of the day, etc.). The specificity of the resistance and the formation of lesions were assessed by challenging *mil1* with *Pseudomonas syringae* pv. *tomato*. *mil1* was not resistant to virulent *P. syringae*, and showed normal HR when challenged with avirulent strains. The level of PR1 expression was monitored after powdery mildew infection. PR1 was not detectable in non infected *mil1* plants, but at 3 dpi, the level of PR1 expression increased to levels about 4 fold higher than wild type. The gene responsible for the *mil1* phenotype was cloned by isolation of sequences flanking the T-DNA insertion, and verified by complementation. MIL1 is a novel protein of 719 aa which is predicted to be membrane anchored and likely possesses two domains: a putative Start domain (involved in lipid-binding in StAR, HD-ZIP and signaling proteins), and a putative PH domain (commonly found in eukaryotic signaling proteins and as constituents of the cytoskeleton). The only other protein found in the databases to have both these domains is a protein of unknown function in *C. elegans*. MIL1 could be a component of cell homeostasis and therefore play a role in the resistance to powdery mildew through cell death.

182 SA signaling: NPR1-dependent and independent pathways

a,bJyoti Shah, bPradeep Kachroo, aAshis Nandi, aDianne Jennings, aMatt Burkhardt, aRachel Buffington and bDaniel F. Klessig

aMCDB Program, Division of Biology, Kansas State University, Ackert Hall, Manhattan, KS 66506, USA; bWaksman Institute & Department of Molecular Biology & Biochemistry, Rutgers University, Piscataway, NJ 08854, USA

Salicylic acid (SA) is an important signal molecule regulating plant defense responses. The Arabidopsis NPR1 gene is a key component of the SA signal transduction pathway. *npr1* mutants (e.g. *npr1-5*) are unable to express the PR (PR-1, PR-2 and PR-5) genes at elevated levels in response to SA. Furthermore, *npr1-5* plants show increased susceptibility to pathogens. However, loss-of-function mutations in NPR1 do not confer complete loss of SA-mediated resistance. The SA-depleted NahG plants are 20-200 fold more susceptible to pathogens than the *npr1* mutant. More recently, resistance against turnip crinkle virus in Arabidopsis has been shown to be SA dependent, but NPR1-independent. Pathogen-induced expression of PR genes in Arabidopsis is also mediated via an NPR1-independent pathway, in addition to the NPR1-dependent pathway. Thus, SA signaling occurs via NPR1-dependent as well as NPR1-independent pathways. The SSI2 gene defines a component of this NPR1-independent defense pathway. Loss-of-function mutations in SSI2 (*ssi2-1* and *ssi2-2*) activate the normally SA-dependent defense responses, independently of NPR1. The *ssi2 npr1-5* double mutant, as well as the *ssi2* NPR1 single mutant, constitutively express the PR genes, accumulate elevated levels of SA, and spontaneously develop lesions. Interestingly, elevated levels of SA are not required for any of the *ssi2-1*-conferred phenotypes. Furthermore, *ssi2-1 nahG* plants are more resistant than SSI2 *nahG* plants to *Pseudomonas syringae* and *Peronospora* spp. Resistance to these pathogens has previously been shown to be SA dependent. Thus, SSI2 appears to function as a negative regulator of this NPR1-independent defense pathway.

183 Characterization of an Arabidopsis-Phytophthora pathosystem

Si-Ammour, A., Roetschi, A., Mauch-Mani, B. and Mauch, F.

University of Fribourg, Plant Biology, 3, route Albert-Gockel, CH-1700 Fribourg, Switzerland

Many oomycetes are devastating plant pathogens causing diseases such as the well-known downy mildews, late blight of potato and seedling damping-off. The molecular analysis of downy mildew diseases has greatly benefited from the Arabidopsis-*Peronospora parasitica* model pathosystem. We have established a model pathosystem for the late blight disease caused by *Phytophthora* species which uses Arabidopsis as host plant and *Phytophthora porri* as pathogen. Our aim is to study the molecular mechanisms of disease development with the goal to learn more about the genetic determinants governing this type of interaction. We will present the initial cytological and biochemical characterization of this new pathosystem. Compatible host-pathogen combinations show the typical characteristics of a facultative biotrophic interaction similar to the agronomically important diseases caused by *P. infestans*. Without causing much symptoms, *P. porri* initially develops a network of intercellular hyphae before the plant tissue collapses in a later stage giving rise to the typical symptoms of the late blight

disease. In incompatible interactions, resistance is expressed in the form of hypersensitive reactions and the formation of papillae and larger cell wall enforcements. Although the accumulation of pathogenesis-related proteins is triggered by inoculation with *P. porri*, these defense proteins do not seem to be essential for the establishment of resistance. *Arabidopsis* mutants compromised in PR-protein accumulation show only a marginally compromised disease resistance. In contrast to other oomycete pathogens such as *Peronospora parasitica*, resistance against *Phytophthora* seems to be largely independent of SA-accumulation and the associated induction of PR-proteins. One of our long term aims is to develop an insertional mutagenesis system for the genetic analysis of *P. porri*. Towards this end, an efficient transformation system was established using *nptII* as a selectable and green fluorescent protein (GFP) as a visual marker. The expression of GFP in transgenic *P. porri* allows the in planta observation of the infection process and will form the basis of future screening systems.

184 ARABIDOPSIS SIGNALLING PATHWAY PROMOTERS IN RESPONSE TO A BACTERIAL PATHOGEN

Karen Sims-Huopaniemi¹, Anders Jensen², John Mundy² and Tapio Palva¹

¹ Department of Biosciences, Division of Genetics, P.O.Box 56, FIN-00014 University of Helsinki, Finland. ² Institute of Molecular Biology, Copenhagen University, Oester Farimagsgade 2A, 1353 Copenhagen K, Denmark

Plants possess a variety of mechanisms to protect themselves against pathogen attack including both pre-formed physical and chemical barriers, and induced responses. An integrated set of defence responses is induced upon recognition of a potential pathogen or its gene products. These include production of antimicrobial compounds such as phytoalexins, cell wall reinforcement, accumulation of pathogenesis-related proteins and an oxidative burst.

Erwinia carotovora subsp. *carotovora* is a non-specific plant pathogenic enterobacterium, which causes soft-rot in many important crop plants including potato. Using *Arabidopsis* as a model plant, our aim is to clarify the role of ethylene, jasmonic acid and salicylic acid pathways in the activation of gene expression during attack by *Erwinia*. Promoters of genes known to be involved in these pathways were cloned using an upstream genome walking method. Transgenic plants carrying fusions of these promoters to the LUC reporter gene were screened for luciferase activity in response to *peh A* and trimers. Those lines with the best-characterised expression were chosen for further T-DNA activation tagging mutagenesis.

185 Genetic and Molecular Analysis of *Arabidopsis* DND Loci

Roger K. Smith, Jr. ^{1,2}, Grace I. Jurkowski ¹, & Andrew F. Bent ^{1,2}

¹. Department of Plant Pathology, University of Wisconsin at Madison, Madison, WI 53706 ². Physiological and Molecular Plant Biology Program, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Two *Arabidopsis* loci, DND1 and DND2 were previously identified. Mutant *dnd* plants have a dwarfed appearance, but are otherwise quite normal in their growth and development. Mutation of each locus induces a "defense, no death" phenotype in which plants exhibit gene-for-gene disease resistance against avirulent *Pseudomonas syringae*, despite the near-complete absence of the hypersensitive response (HR). Macroscopic and

microscopic studies have shown that most sets of *dnd* plants are not lesion-mimics, but in rare instances we have observed a punctate, non-spreading lesion-mimic phenotype that is elicited by unknown environmental factors. The *dnd* mutants also exhibit constitutive systemic resistance and elevated levels of salicylic acid. These mutants are a useful tool for dissection of HR cell death, SAR and related disease resistance pathways. Positional cloning revealed that *dnd1* mutant phenotypes are caused by disruption of a cyclic nucleotide-gated ion channel (Clough et al., submitted). No significant shifts in *DND1* mRNA levels were observed in leaf tissue 24 hours after plants were treated with virulent or avirulent *P. syringae*, or 48 hours after treatment with salicylic acid. To further study the loss of HR in *dnd* mutants, their response to other elicitors of cell death is being examined. Reduced sensitivity to fumonisin was observed, suggesting that *dnd* plants display a more generalized suppression of cell death. Progress with other elicitors will be reported. Control of reactive oxygen species (ROS) is being examined by diaminobenzidine staining and by northern blot analysis of ROS scavengers in *dnd* plants. Double mutants are being constructed to place *dnd1* with respect to other *Arabidopsis* genes involved in defense. Preliminary results regarding *dnd1-npr1* and *dnd1-ndr1* double mutants will be presented.

186 A cytological comparison between host and non-host resistance to fungal pathogens in *Arabidopsis*

Monica Stein and Shauna Somerville

Dept. of Biological Sciences, Gilbert Hall, Stanford University 94305: Dept of Plant Biology, Carnegie Institute of Washington, 260 Panama St., Stanford, CA 94305

Plants are constantly being exposed to a wide variety of pathogens. However, a given plant species is host to only a subset of these pathogens. Resistance to pathogens outside this subset is termed non-host resistance. Non-host resistance is thought to be multigenic, non-specific, and durable. In contrast, the resistance of certain genotypes of an otherwise susceptible species to a pathogen is termed host resistance. This type of resistance often follows gene for gene interactions and is typically short lived in the field. To study differences between these two resistances, host and non-host resistance was compared cytologically.

Arabidopsis is a host to the powdery mildew *Erysiphe cichoracearum*, and a non-host to *Blumeria graminis* f.sp. *hordei*, a pathogen of barley. Two *A. thaliana* isolines, derived from a cross between Col and Kas and differing at a locus that conferred resistance to *E. cichoracearum*, were infected with both pathogens. Resistance to *E. cichoracearum* occurred late, at the onset of conidiation (5dpi). The response to *E. cichoracearum* infection included the formation of papillae and rarely the formation of auto-fluorescent compounds. Interestingly, no cell death was associated with the resistance. Resistance to *Blumeria graminis* f.sp. *hordei* occurred around 1dpi. Non-host resistance was correlated with the formation of papillae, strong callose accumulation in both epidermal and mesophyll cells, presence of auto-fluorescent compounds, hydrogen peroxide accumulation and some microscopic cell death. While most spores were arrested at penetration, 6% of spores formed haustoria and produced branched hyphae. Cytological differences between host and non-host interactions may be used as markers to screen for genes involved in *Arabidopsis* non-host resistance to *Blumeria graminis* f.sp. *hordei*.

187 Dwarfing Variant with Altered Disease Response

Trevor L. Stokes, Eric J. Richards and Barbara N. Kunkel

Washington University

We previously identified a dwarfing mutant, *bal*, that arose in a *ddm1* DNA hypomethylation background. Though stable under normal growth conditions, the *bal* mutant can revert at a high frequency suggesting that the phenotype is due to epigenetic rather than genetic alterations. Several lines of evidence lead us to suspect that the *bal* mutant has an altered pathogen response. First, the *bal* mutant maps to an RPP5-like gene cluster on chromosome 4. RPP5 (Resistance to *Peronospora parasitica* 5) is involved in upstream events of pathogen perception. It is known that ectopically expressing certain R genes confers activation of several components of the resistance pathway. Second, we detect a 6.5 Kb message from the region the level of which positively correlates with the severity of the dwarfing phenotype. Third, *bal* resembles the previously identified mutant, *cpr1*, at the gross morphological level. Thus, we assayed defense responses in *bal* and determined that *bal* has a constitutive disease response. The *bal* mutant constitutively expresses PR genes, making it a *cpr* mutant. When challenged with *Pseudomonas syringae*, bacterial growth is significantly reduced in the *bal* mutant. In contrast, bacterial growth is near wild-type levels in the *bal* revertants. The *bal* phenotype can also be suppressed in the presence of *nahG*, consistent with the idea that *bal* is involved in an SA dependent resistance response. Using molecular markers tightly linked to the BAL locus, we verified that BAL maps very closely to CPR1. Genetic interactions between *bal* and *cpr1* will be discussed.

188 Physiological and Genetic Analyses of *edr5*, an Arabidopsis Enhanced Disease Resistance Mutant

Lisa M. Stutius*, Sigrid M. Volko, and Frederick M. Ausubel

*Harvard College, Cambridge, MA 02138; Dept. of Genetics, Harvard Medical School, Boston, MA 02115; and Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

An Arabidopsis mutant, *edr5-1* (corresponding to a new Arabidopsis gene, EDR5), displays enhanced disease resistance to bacterial and fungal pathogens in the absence of constitutive PR gene expression. *edr5-1* was isolated by virtue of reduced disease symptoms and growth of *Pseudomonas syringae* pv. *maculicola* (Psm) ES43262 (Volko, unpublished data). It also displays altered disease symptoms when challenged with *Erysiphe orontii*, an obligate fungal pathogen. Higher induced levels of PR gene expression and callose production are seen in response to PsmES4326 and *E. orontii*, respectively, in the *edr5* mutant. The *edr5* phenotype is conferred by a recessive mutation mapping to chromosome 4. Presently, at least five EDR genes have been isolated, of which *edr1* is the best understood. The recessive *edr1-1* mutation confers resistance to a variety of fungal and bacterial pathogens and correlates with a more rapid induction of defense responses, a phenotype strikingly similar to *edr5*. However, Northern blot analyses reveal differences in the timing and level of PR gene induction in response to *E. orontii* between *edr1* and *edr5* plants (S. Volko, unpublished data). Based on their similar phenotypes, *edr5* and *edr1* may lie in the same genetic pathway or have similar functions. Since mutating either gene results in enhanced disease resistance, the wild-type functions

of *edr5* and *edr1* are likely to be that of repressors for defense responses against a variety of pathogens. Mapping, cloning, and further characterization of the *edr5* mutation will elucidate the genetic and biological role of the *EDR5* gene in regulating plant defense.

1. Frye, C.A. and Innes, R.W. (1998) *Plant Cell*, 10, 947-956.
2. Volko, S.M., Boller, T., and Ausubel F.M. (1998) *Genetics*, 139, 537-548.

189 Specific Recognition of a Bacterial Protein by Arabidopsis Requires Both an NBS-LRR Protein and a Kinase

Swiderski, MR, Ade, J, Merritt, PM, Nobuta, K, and Innes, RW
Department of Biology, Indiana University, Bloomington, IN 47405

Disease resistance in plants is often mediated by "gene-for-gene" interactions involving specific plant resistance (R) genes and pathogen avirulence (*avr*) genes. The molecular cloning of R genes has revealed four classes of proteins. The largest class is defined by the presence of a nucleotide binding site (NBS) and leucine rich repeats (LRRs). Because LRRs are known to function as protein:protein interaction domains, it has been widely speculated that NBS-LRR proteins may function as receptors for *avr* protein ligands. Direct physical evidence for this model is still lacking, however.

We are investigating the molecular basis of pathogen recognition in Arabidopsis, in particular, recognition of the pathogen protein *AvrPphB*, from the bacterium *Pseudomonas syringae*. We have identified via mutant screens at least 5 genes that are required for full resistance to *P. syringae* strains that express *avrPphB*. Two of these genes, *RPS5* and *PBS1* appear to be required specifically for *avrPphB* recognition as null mutations in these genes do not affect recognition of other pathogens. The remaining three genes, *PBS2*, *PBS3* and *NDR1* are believed to function in shared signal transduction steps as mutations in these affect multiple R gene pathways.

We have previously reported that *RPS5* encodes an NBS-LRR protein. We have recently determined that *PBS1* encodes a kinase with possible serine/threonine/tyrosine specificity. *PBS1* belongs to the same subfamily of kinases as the *Pto* and *Pti1* kinases from tomato. The *Pto* kinase is required for recognition of the *avrPto* protein, along with an NBS-LRR protein called *Prf*. This suggests that most, and perhaps all NBS-LRR proteins require a kinase for functional recognition of pathogen proteins. If so, what is the role of each in pathogen recognition? Strong evolutionary arguments suggest that NBS-LRR proteins should function as the specific receptor; however, in the *Pto/Prf* system, the *Pto* kinase interacts with *avrPto*, at least in a yeast two-hybrid assay. Thus, the precise role of NBS-LRR proteins in pathogen recognition remains obscure. Several models and means for testing them will be discussed.

190 *PBS1* kinase - one more piece in *AvrPphB* recognition puzzle

Michal R. Swiderski, David A. Hubert and Roger W. Innes
Department of Biology, Indiana University, Bloomington, IN 47405

The *pbs1* mutant of *Arabidopsis thaliana* is characterized by susceptibility to *Pseudomonas syringae* DC 3000/*AvrPphB* and lack of a hypersensitive response (HR) elicited by the *AvrPphB* avirulence protein. Positional mapping identified two BACs, F20L19 and F7J12 on chromosome V, as possibly containing the gene. Detailed analysis of the F7J12 region in *pbs1* mutant plants identified a 1.5 kb deletion in a kinase gene in

the pbs1-1 (a fast neutron induced allele) and G-A missense mutation in the kinase domain of the same gene in the pbs1-2 (an EMS induced allele). This substitution leads to a change of a conservative Gly into Arg in subdomain VIII of the kinase domain. Transient expression of PBS1 in pbs1-1 plants stably transformed with avrPphB elicited a strong hypersensitive reaction. PBS1 gene spans 4.5 kb and contains 4 introns. The PBS1 protein contains characteristics of Serine/Threonine kinases and shows 65% similarity to *Apk1a* kinase. The same level of similarity is observed to PTO and PTII kinases from tomato. Another gene required for specific resistance against DC3000/AvrPphB, RPS5, has been characterized earlier, thus two genes are required for specific recognition of AvrPphB. This resembles PTO-mediated resistance in tomato, which also requires a second gene, Prf, to confer specific recognition of *P. syringae* pv tomato carrying avrPto. Both, RPS5 and PRF, belong to the NBS-LRR class of resistance proteins. Yeast two-hybrid screen identified one specific interacting protein, PBS1-AsF1, with weak homology to myosin. The biological relevance of this interaction has yet to be determined. Current progress in characterization of PBS1 protein will be presented and its possible function in plant resistance and AvrPphB-induced cell death will be discussed.

191 Identification of a MAP3 kinase that negatively regulates defense responses in *Arabidopsis*

Dingzhong Tang, Catherine A. Frye, Roger W. Innes

Department of Biology, Indiana University, Bloomington, IN

We have identified an *Arabidopsis* mutant that displays enhanced disease resistance (*edr1*) to the powdery mildew pathogen, *Erysiphe cichoracearum*. Multiple defense responses are induced more rapidly in *edr1* plants than in wild type Col-0 plants when infected with *E. cichoracearum*. Autofluorescent compounds and callose accumulated in *edr1* leaves and cell death occurred after inoculation. EDR1 was isolated by a positional cloning approach. The predicted EDR1 protein is a member of the mitogen activated protein (MAP) kinase kinase kinase family and shows high similarity to CTR1, a negative regulator of the ethylene signal transduction pathway. The recessive nature of the resistance in *edr1* and the high similarity to CTR1 suggest EDR1 may function as a negative regulator of specific defense responses. The EDR1 gene is well conserved among both monocots and dicots, suggesting that it may function in a similar way in all higher plants. The EDR1 is constitutively expressed, but mildly induced by *E. cichoracearum*. Although EDR1 is similar to CTR1, the *edr1* mutant does not constitutively express ethylene-regulated genes, Chi-B and Hevin-like gene, and no interaction has been found between EDR1 and the ethylene receptors, ETR1, ETR2, ERS1, ERS2 and Ein4 by yeast two hybrid assay. Furthermore, the ethylene insensitive mutant *ein2* does not suppress the *edr1* phenotype, indicating that the EDR1 protein does not function in the ethylene response pathway. However, *edr1* mutants shown enhanced senescence compared to Col-0 wild-type plants when exposed in ethylene, indicating the *edr1* is more sensitive to ethylene. The *npr1* mutation partially suppresses *edr1*-mediated resistance to *E. cichoracearum*, suggesting that the SA signal transduction pathway contributes to the *edr1* phenotype.

192 Microarray analysis of the compatible interaction between *Arabidopsis* and *Pseudomonas syringae* pv. tomato DC3000

Roger Thilmony, Julie Zwiesler-Vollick, Robert Schaffer, Jeff Landgraf, Ellen Wisman and Sheng Yang He

Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

We are investigating the susceptible interaction between *Arabidopsis* and the virulent bacterial pathogen *Pseudomonas syringae* pv. tomato strain DC3000 (Pst DC3000). The Pst DC3000 *hrp* gene cluster is required for pathogenicity in susceptible hosts and encodes the components of a type III protein secretion system responsible for the delivery of avirulence and virulence proteins to the plant host cell. We have utilized the *Arabidopsis* Functional Genomics Consortium (AFGC) microarray to compare the *Arabidopsis* response to Pst DC3000 and a Pst DC3000 *hrpS* - non-pathogenic mutant. From the AFGC microarray analysis, more than 300 *hrp*-dependent, Pst DC3000-regulated genes were identified. In an effort to further characterize the expression of these genes, we have created our own microarray slide containing these cDNA clones. We are currently examining the specificity of their differential expression using NahG transgenic plants, *npr1*, *coi1* and *ein2* mutant plants inoculated with Pst DC3000 to determine whether the differentially regulated genes are salicylic acid, jasmonic acid and/or ethylene dependent. We also are investigating whether individual bacterial proteins (putative virulence factors) are capable of inducing differential expression of subsets of the Pst DC3000-regulated genes. Our goal is to identify those *Arabidopsis* genes that are specifically regulated by virulence proteins secreted via the type III secretion system.

193 HERITABILITY OF INDUCED SYSTEMIC RESISTANCE IN ARABIDOPSIS

Ton Jurriaan, Davison Sylke, Vos de Martin, Van Loon Leendert C., Pieterse Corné M.J. Section of Phytopathology, Utrecht University, The Netherlands

Selected nonpathogenic rhizobacteria with biocontrol activity are able to elicit an induced systemic resistance (ISR) response in plants. Different ecotypes of *Arabidopsis thaliana* were screened for their potential to express rhizobacteria-mediated ISR against infection by *Pseudomonas syringae* pv. tomato DC3000 (Pst). Of the 10 ecotypes tested, ecotypes RLD and Ws did not develop ISR after treatment of the roots with the nonpathogenic *Pseudomonas fluorescens* strain WCS417r. This WCS417r-nonresponsive phenotype was correlated with a remarkably high level of susceptibility to Pst infection and relatively low sensitivity to the plant hormone ethylene. A genetic analysis of a cross between the WS417r-responsive ecotype Col and the WCS417r-nonresponsive ecotype RLD revealed that the potential to express ISR, basal resistance against Pst and ethylene sensitivity cosegregate in a 3 : 1 fashion, suggesting that the three characteristics are monogenically inherited and genetically linked. Neither the responsiveness to WCS417r nor the relatively high level of basal resistance against Pst were complemented in the F1 progeny of a cross between RLD and Ws, indicating that both ecotypes lack the same genetic determinant. The corresponding locus, designated ISR1, was mapped on chromosome 3 between markers GL1 and B4. The presented data suggest that ecotypes RLD and Ws are affected in an ethylene response gene that is involved in ISR signalling and basal resistance against Pst. To further elucidate the association between basal resistance and induced resistance, a set of *Arabidopsis* mutants exhibiting enhanced disease susceptibility to *P. syringae* pv. *maculicola* was tested for the potential to express both rhizobacteria-mediated ISR and pathogen-induced systemic acquired resistance (SAR).

Out of 11 eds-mutants tested, three failed to express WCS417r-mediated ISR and two failed to express pathogen-induced SAR. Currently, the ISR- and SAR-impaired eds-mutants are tested for their production and sensitivity to the plant hormones ethylene, jasmonic acid and salicylic acid.

194 Dissecting Plant-Insect Interactions

H. Vogel, J. Kroymann, V. Lambrix, T. Mitchell-Olds

Max-Planck-Institute of Chemical Ecology

Plants deploy diverse biochemical and molecular defenses against herbivores and pathogens. In addition to preformed chemical and physical barriers, inducible defenses contribute to plant protection.

Herbivory induces many wound-responsive genes both locally and systemically, and involves a lipid-mediated signaling cascade, preceded or mediated by phosphorylation events and by transient increase in ion fluxes and cytosolic Ca²⁺, leading to the accumulation of defense proteins. Signaling molecules include jasmonic acid, abscisic acid, ethylene, systemin, and oligosaccharides.

To dissect the complex interactions between plants and their insect herbivores, we focus on a model system consisting of *Arabidopsis thaliana* (and related species of the genus *Arabis*), and insects such as *Plutella xylostella* (Diamondback Moth) and *Spodoptera littoralis*. The following approaches are employed:

1. Screen for mutants with altered sensitivity against the specialist *Plutella* and/or the generalist *Spodoptera*
2. QTL mapping to exploit natural occurring variation in different *A. thaliana* ecotypes
3. Positional cloning of candidate genes
4. Identify defense-related genes by differential display and cDNA subtraction methods, followed by reverse genetics
5. Once identified, the expression profiles of herbivory-related genes from both *Arabidopsis* and *Arabis* are monitored by membrane-based macroarray approaches. This allows simultaneous analysis of large subsets of genes, which is a prerequisite to understanding the inherent complexity of signal transduction pathways and gene regulation involved in plant-insect interactions.

195 Powdery mildew-resistant *Arabidopsis* mutants

John Vogel and Shauna Somerville

Carnegie Institution of Washington

A compatible interaction between a plant and a pathogen is the result of a complex interplay between many factors of both plant and pathogen origin. Our objective is to identify host factors involved in this interaction. To this end, we have identified 26 recessive *Arabidopsis* mutants that do not support normal growth of the powdery mildew pathogen, *Erysiphe cichoracearum*. Six loci, designated powdery mildew resistant 1-6 (*pmr1-6*), are defined by this collection. The gene corresponding to *pmr6* has been cloned and belongs to the pectate lyase gene family. *PMR6* is predicted to be attached to the plasma membrane by a novel C-terminal domain containing a GPI anchor. This domain is unique among the pectate lyase-like genes in *Arabidopsis* suggesting that it may be important for the interaction with powdery mildew. Significantly, *pmr6* resistance does not require salicylic acid or ethylene perception. *pmr1-pmr4* do not constitutively

accumulate elevated levels of PR1 or PDF1.2 mRNA, indicating that resistance is not simply due to constitutive activation of the salicylic acid- or jasmonic acid-dependent defense pathways. Further northern analyses revealed that *pmr1* and *pmr4* accumulate slightly higher levels of PR1 mRNA than wild type after infection with powdery mildew. To test the specificity of the resistance, the mutants were challenged with other pathogens including *Pseudomonas syringae*, *Peronospora parasitica* and *Erysiphe orontii*. Surprisingly, one mutant, *pmr1*, was susceptible to *E. orontii*, a very closely related powdery mildew, suggesting a very specific resistance mechanism is operating in this case. Another mutant, *pmr4*, was resistant to *P. parasitica* indicating that this resistance is more generalized. Most of the mutants are pleiotropic indicating that the genes affected play roles in normal plant growth and development. For example, *pmr1* has a pollen defect indicating that PMR1 normally plays a role in pollen tube growth or guidance. Thus, we have identified a novel collection of mutants affecting genes required for a compatible interaction between a plant and a biotrophic pathogen. These mutants can also be considered a novel and potentially durable form of disease resistance. Supported by NIH fellowship F32 GN19499-01 and the Carnegie Institution of Washington.

196 Isolation Of Plant Host Factors Required For Geminivirus Infection

Doreen H. Ware, Kenneth J. Buckley, Keith R. Davis,

Department of Plant Biology and the Plant Biotechnology Center, Ohio State University

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). Overexpression of ORF L4 in transgenic *Arabidopsis* mimic symptoms observed during viral infection and include novel phenotypes. Viral mutations of ORF L4 were generated at positions of identity in two strains where the L4 proteins only show an overall 47% amino acid sequence identity. Viral mutations of ORF L4 lead to reduced symptom development in infected plants. These results suggest that ORF L4 is a major symptom determinant in *Arabidopsis*. A yeast two-hybrid screen was initiated using BCTV-CFH ORF L4. A partial cDNA clone, YC58, was identified and tested positive against the ORF L4 of the second BCTV virus, Logan. Given that there is only 47% sequence identity between the L4 proteins of the two strains of BCTV, this strongly suggests that this interaction is functionally relevant. YC58, a serine threonine kinase, is a homologue of 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, morphogenesis and mitogenesis. In *C. elegans*, *Drosophila*, and vertebrates, GSK3 is a 1 or 2 gene family; however, in *Arabidopsis*, shaggy kinases (ASK) comprise a family of at least 10 members (Dornelas, 1998). In this poster, we present data on the expression pattern of two ASK members and the functional analysis of the two genes by overexpression.

197 Identification of Pathogen-Inducible ERF-Like Sequences

Randall F. Warren and Jian-Min Zhou

Kansas State University, Dept. of Plant Pathology, 4024 Throckmorton Plant Sciences Center

Various members of the ethylene-responsive element binding factor (ERF) gene family are induced by diverse pathogens in a variety of plants. For example, transcripts from the tomato genes Pti4 and Pti5 accumulate after infection by *Pseudomonas syringae* pv tomato. Furthermore, these gene products interact with Pto, a resistance gene product, according to yeast two-hybrid assays. ERFs appear unique to plants, are hypothesized to function as transcription factors, and have been implicated in salicylic acid (SA), jasmonic acid (JA), and ethylene signaling. We have used a DNA macroarray to identify *Arabidopsis* ERFs that potentially exhibit altered expression after infection with *P. syringae* pv tomato strain DC3000 carrying avrB. DNA from 20 independent Expressed Sequence Tags (ESTs) that exhibited sequence similarity to the DNA binding domain of ERFs was blotted in duplicate and analyzed with reverse transcribed probes derived from mRNA isolated from either pathogen-infected or mock-inoculated tissue. Differential expression was present for 10 of the EST sequences. These EST sequences were hybridized to Northern blots and signals from six of the ESTs were detectable on autoradiograms and exhibited a differential response. Five ERF-like transcripts appear to be upregulated by pathogen infection and one appears to be repressed. In addition to analyzing the effect of different avirulent and virulent bacterial strains on transcript accumulation, we are examining their responses to SA, JA, and ethylene. We are also determining if various mutations that induce or repress pathogen defense responses in *Arabidopsis* affect transcript accumulation of the six ERF-like sequences.

198 Genetic Screen to Identify *Arabidopsis* Mutants Which Overcome a Nitric Oxide Synthase Inhibitor Block of the Hypersensitive Response

Cathy K. Worley, Laura E. Maliszewski, Joshua H. Hubner, and Allan D. Shapiro

University of Delaware, 152 Townsend Hall, Newark, DE 19717

12,751 EMS-mutagenized M2 *Arabidopsis* plants were screened for their ability to overcome an L-NNA (a nitric oxide synthase inhibitor) block of the hypersensitive response (HR) elicited by inoculation with *Pseudomonas syringae* pv. *glycinea* Race 5 carrying avrRpm1. In wild-type Columbia accession *Arabidopsis*, 85% of the leaves exhibited a HR one day post-inoculation with 1×10^8 bacteria/ml, but only 20% of the leaves exhibited a HR if 200 micromolar L-NNA was included in the inoculum. L-NNA had no effect on bacteria viability. For the screen, 5 leaves per plant were inoculated with bacteria plus L-NNA, and plants which exhibited HRs on at least 80% of the leaves were kept as positives. 793 plants tested positive. We will test 15 M3 from each positive to verify phenotypes. 61 lines have been re-tested in the M3 generation thus far, and 4 re-tested as bonafide mutants which appear to be homozygous for the mutation. Pending mapping and complementation information, we are calling them hdn-1, hdn-2, hdn-3 and hdn-4 for HR Despite NOS inhibitor. hdn-2 exhibits a lesion mimic phenotype. 9 more lines segregated ~3:1 for a positive phenotype suggesting the original isolates were heterozygous for a dominant mutation. For these lines, we will test 15 M4 from at least 9 sibs to isolate a homozygous line. We expect to identify genes involved in the

regulation of nitric oxide signaling or parallel pathways which can bypass the requirement for nitric oxide in eliciting the HR.

199 *Arabidopsis thaliana* ethylene-mediated root hair development influences susceptibility to the sugar-beet cyst nematode by modulating root attractiveness
Martin J. E. Wubben¹, Hong Su¹, Steven R. Rodermel^{1,3}, and Thomas J. Baum^{2,3}
¹Interdepartmental Genetics Program, ²Department of Plant Pathology, ³Botany Department, Iowa State University, Ames, IA 50011

We previously identified the recessive *Arabidopsis thaliana* *cst2* (altered susceptibility to cyst nematode) mutant that is hypersusceptible to the sugarbeet cyst nematode, *Heterodera schachtii*. Further characterization of *cst2* revealed that it exhibits shorter roots, increased root hair length and density, and deformation of root epidermal cells. Elevated ethylene concentrations are known to inhibit root elongation and promote root hair development in *A. thaliana*, therefore, the possible role of ethylene in the *cst2* phenotypes was studied. AVG, an inhibitor of ethylene production, was able to rescue all *cst2* phenotypes and decreased wild-type susceptibility and root hair length and density. AgNO₃, an inhibitor of ethylene perception, reduced *cst2* root hair length and density to that of wild-type. The ethylene precursor ACC increased root hair length and density and susceptibility in wild-type plants, phenocopying *cst2*. These results suggested that an alteration in ethylene signal transduction in the *cst2* mutant was responsible for the observed phenotypes. To further examine the effects of ethylene on plant susceptibility to *H. schachtii*, we determined the susceptibility of known ethylene mutants. Mutants exhibiting an increased ethylene response resulting in more and longer root hairs were hypersusceptible to *H. schachtii*. Mutants that were ethylene insensitive and showed fewer and/or shorter root hairs were less susceptible. Nematode attraction assays using root exudate from *cst2* and *eto3* (ethylene-overproducing) roots revealed these mutant exudates were more attractive to *H. schachtii* juveniles than wild-type exudate. While it has been previously observed that infective juveniles of cyst nematodes are attracted to the zone of elongation of host roots prior to penetration, it is unknown which event in plant development serves to provide the attractant. Our results show that ethylene-mediated root hair initiation in the zone of elongation is positively correlated with the release of an unknown nematode attractant. *cst2* has been mapped to the bottom of chromosome 1.

200 A new type of plant disease resistance gene at the RPW8 locus controls powdery mildew diseases of *Arabidopsis*

Shunyuan Xiao, Simon Ellwood, Ozer Calis, Tianxian Li, Mark Coleman, John Turner
School of Biological Sciences, University of East Anglia, Norwich, England

Plant resistance genes couple the recognition of specific pathogens to the induction of non-specific defences that localise the invader at the point of infection. More than twenty plant resistance genes have been cloned and characterised. With the exception of *Pto*, which is a protein kinase, these encode proteins with leucine rich repeats (LRRs) and with different combinations of motifs that have similarity to a nucleotide binding site (NB), a leucine zipper, the Toll/interleukin receptor, a transmembrane domain, and a protein kinase. Most of the isolated resistance genes are of the NB-LRR type, and many more similar genes have been detected in *Arabidopsis thaliana* and other species. We

have used *A. thaliana* as a model to characterise genes for resistance to the powdery mildews, which cause severe losses on a wide range of crop species. Resistance of *A. thaliana* accession Ms0 to the powdery mildew pathogen, *E. cichoracearum* is regulated at the RESISTANCE TO POWDERY MILDEW8 (RPW8) locus on chromosome 3. We show that RPW8 comprises two related genes, RPW8.1 and RPW8.2, which independently control resistance to four powdery mildew diseases, and encode a novel type of disease resistance protein.

201 Reactive Oxygen, NDR1 and NPR1 in Arabidopsis Disease Resistance Signaling
Zhang Chu, Shapiro Allan D.

Department of Plant and Soil Sciences, University of Delaware

Mutants in the NDR1 gene are compromised in resistance to multiple strains of *Pseudomonas syringae* and *Peronospora parasitica*. The *ndr1* mutants are also affected in specific processes correlated with disease resistance in a pathogen strain-specific manner. Previous work has suggested that NDR1 acts downstream of the oxidative burst and upstream of induced salicylic acid (SA) biosynthesis. This model would predict that levels of reactive oxygen production seen early in the response to avirulent pathogen should not be affected by the *ndr1* mutation. A burst of hydrogen peroxide production was seen between 2 and 5 hours post-inoculation with *P. syringae* pv. tomato DC3000 carrying *avrB* or *avrRpt2*. No significant differences were noted between *ndr1-1* and the Columbia parental. We have made an *ndr1-1/npr1-2* double mutant. The *npr1* mutation was not found to have any effects on the hypersensitive response (HR) either in the single or the double mutant. As such we conclude that the signaling pathway downstream of *npr1* and any feedback affected by the *npr1* mutation do not contribute to the HR. Analysis of PR-1, PR-2 and PR-5 induction in single and double mutants in response to both virulent and avirulent *P. syringae* showed that cell death and SA make independent contribution to PR gene induction.

202 Characterization of novel virulence factors of *Pseudomonas syringae* pv. tomato strain DC3000

Julie Zwiesler-Vollick, Wensheng Wei, Anne E. Plovanich-Jones, Xin Rong, and Sheng Yang He

Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48823

We are studying the pathogenic interaction between *Arabidopsis thaliana* and the bacterial pathogen *Pseudomonas syringae* pv. tomato (Pst) DC3000. This system has been utilized effectively for the study of the events which lead to a resistant response. However, our understanding of the factors involved in Pst DC3000 virulence is limited. Currently, there are only two known factors which play a role in pathogenicity and virulence. First, the phytotoxin coronatine aids in the development of chlorotic lesions and may play a role in suppressing the host defense response during infection of *A. thaliana* by Pst DC3000. Second, pathogenicity of Pst DC3000 is dependent on the presence of a group of genes known as the *hrp* (hypersensitive response and pathogenicity) gene cluster. This gene cluster encodes a functional type III protein secretion system and is a method for the delivery of bacterial proteins into the eukaryotic host cells. While this secretion system is required for pathogenesis, it does not appear to

contain all the virulence factors of Pst DC3000. We have utilized a transposon mutagenesis strategy to discover additional virulence factors of Pst DC3000. A mini-Tn5- β -Glucuronidase (GUS) construct was utilized to generate both insertional mutations and GUS reporter gene fusions concomitantly. The known virulence and pathogenicity factors of Pst DC3000 are expressed in vitro in minimal media. Tn5 mutants were analyzed for GUS expression when grown in rich media and minimal media. Further analysis of those mutants which did not express GUS in rich media but showed GUS expression in minimal media was carried out. Seven mutants showed reduced virulence when infiltrated into the susceptible ecotype Columbia gl1 mutant. These mutants were still able to trigger a non-host hypersensitive response (HR) in tobacco. This indicates that the mutation does not affect the hrp gene cluster. Thus these are probably previously undescribed virulence factors. Characterization of the phenotype, and growth in planta of these mutants will be presented. Also, the sequences of the mutated genes will be determined and the possible role of these genes in virulence will be described.

203 Identification of New Loci Involved In Ethylene Response Using Non Saturating Concentrations of ACC

Alonso J.M., Stepanova A.N., Solano R. and Ecker J.R.

University of Pennsylvania, Dept. of Biology

Using the typical triple response assay and a novel screening based on non-saturating concentrations of ACC we have identified a set of new ethylene-insensitive mutants. In contrast to previously identified mutants, the mutants described herein show a normal or near normal response to saturating concentrations of ethylene but are clearly insensitive to low concentrations of the hormone. *wei1*, *wei2*, and *wei3* represent new ethylene loci, whereas *wei4* and *wei5* map in regions near *ERS1* and *EIL1*, respectively. Although the two latter genes have been implicated in ethylene signaling using transgenic plants, until now no mutants in these loci have been described.

Genetic characterization of *wei4* indicates that it is a dominant mutation that can be suppressed by *ctr1*. *wei4* maps to the bottom of chromosome 2. Sequence analysis of the *ERS1* locus in the *wei4* plants reveals a missense mutation. This mutation affects a highly conserved amino acid and is different from those previously identified in other ethylene receptor genes. *wei5* maps to the middle of chromosome 2 next to the *ER* and *EIL1* loci. Complementation analysis indicates that *wei5* and *ein3-1* mutations have synergistic effects. Epistatic analysis shows that *wei5* can partially suppress *ctr1-1*. Sequencing of the *EIL1* locus in the *wei5* plants reveals an insertion of 4 nt that results in a frameshift and a premature termination of the protein. Another allele of *EIL1*, *eil1-1*, was isolated by a PCR-based approach. Double mutants *ein3-1 eil1-1* and *ein3-1 eil1-2* show strong ethylene insensitivity in etiolated seedlings, similar to that of the *ein2-5* mutant, implying that other EILs may not be involved in ethylene signaling at this developmental stage. Map positions and epistatic analysis of the *wei1*, *wei2* and *wei3* mutants are currently under investigation.

204 Structural and Functional Characterization of ARF11/IAA22

Arima, K., Overvoorde, P.J., and Theologis, A.

Plant Gene Expression Center, 800 Buchanan, Albany, CA 94710

The proteins encoded by the auxin-regulated Aux/IAA genes are short-lived, nuclear-localized, and have the capacity to form homo- and hetero-dimers through domain III, one of four conserved motifs found in these proteins. Using IAA1 as bait in a two-hybrid screen, new members of the Aux/IAA protein family (IAA16-20) and proteins that contain the Aux/IAA-like domains III and IV (IAA21-25) were isolated. Subsequent analysis revealed that IAA21-25 are members of the ARF family of transcription factors that can bind to the auxin-responsive elements located in the promoters of many early auxin-inducible genes, including members of the Aux/IAA gene family. IAA21/23/25 represent ARF7 and IAA24 corresponds to ARF5/MP. IAA22 is a new member of the ARF family named ARF11. The ARFs are encoded by at least 16 genes in Arabidopsis and their mRNA levels are not induced by auxin or cycloheximide. The ARF11 protein has a predicted MW of 120 Kda and shares structural and functional similarity with other members of the ARF protein family. ARF11 is encoded by 12 exons and contains the conserved VP1-B3 DNA-binding domain at its N-terminus and the Aux/IAA-like domains III and IV present in most ARFs at its C-terminus. Like ARF5-8, the ARF11 protein contains a Q-rich rich region in the middle of the protein. The ability of ARF11 to interact with the auxin-responsive elements ER7 and ER9 is shown using a yeast one-hybrid assay. This approach has also been used to examine the DNA binding affinity of other ARFs to the ER7 and ER9 binding sites. To determine the function of the ARF11 protein during plant growth and development we are constructing anti-sense and overexpression lines as well identifying T-DNA insertion mutants. Several lines containing T-DNA insertions have been identified and are currently being characterized. The possible role of ARF11 in auxin signaling will be discussed.

205 Cloning the age1 mutant using Denatured High Pressure Liquid Chromatography (DHPLC) technology

Qixin Bei¹, Yutaka Oono², Athanasios Theologis¹

¹Plant Gene Expression Center, 800 Buchanan Street, Albany, 94710 California, ²Japan Atomic Energy Research Institute, Takasaki 370-1292, Japan

Age1 is an auxin hypersensitive Arabidopsis mutant with altered auxin-regulated gene expression (Oono et al. (1998) Plant Cell, 10:1649-1662). It was identified using a reporter gene (GUS) driven by the auxin responsive domains AuxRD A and B of the early PS-IAA4/5 gene. The reporter gene expression is detected at 10-100 fold lower auxin concentration (10^{-8} to 10^{-9} M) in the age1 than in the wild type. We are using DHPLC technology for mapping and cloning this mutant.

DHPLC is a method developed recently to detect single nucleotide polymorphism (SNP), as well as short insertions and deletions. The method is based on the principle that if there is a SNP between two DNA fragments, denaturation and reannealing of the fragments allow the formation of heteroduplexes with unpaired bases resulting from the SNP. This heterogeneity between the homo- and hetero-deplexes can be resolved by DHPLC. In addition mapping of age1 mutant has been facilitated by DHPLC markers. The markers are generated by converting traditional CAPS markers to DHPLC markers. Primers are designed to nest the polymorphic sites in PCR products of 200-300 bp followed by DHPLC.

For identifying the EMS induced mutation in AGE1 gene, overlapping PCR products are synthesized across the mapped region (150 Kb region at the top of chromosome 1) using

wild type and age1 genomic DNA. The primers with Tm of 56C are selected with the Primer3 program. After PCR amplification the products from the wild type and age1 DNA are mixed together. The mixture is denatured at 95C and allowed to anneal from 95C to 65C in 30 minutes. The reannealed PCR-products are injected into DHPLC (Transgenomic-WAVE) and checked for heteroduplexes at appropriate denaturing temperature.

206 Leaf anatomy characterization of the Arabidopsis mutant, era 1-2

Siobhan Brady, Dario Bonetta and Peter McCourt

Department of Botany, University of Toronto

The Arabidopsis thaliana era 1-2 mutant has pleiotropic phenotypes throughout its development. At the germination level, its seeds display an enhanced response to exogenous abscisic acid. During its vegetative phase, leaf blade margins do not curve downward and leaves appear broadened. The dome of the inflorescence meristem is significantly larger than wild type and cell number within the dome is increased almost 3-fold as compared to wild type. In the present study it was determined that the broad leaf phenotype is most likely the result of an increase in cell division and not in cell expansion. The leaves of era 1-2 have an increased thickness, an increase in adaxial epidermal cell number, more palisade mesophyll cell layers, a decrease in intercellular air space, and no significant difference in the ratio of length to width of palisade mesophyll cells. The ERA1 gene codes for a protein farnesyltransferase and these results support a role of the farnesyltransferase in cell cycling. Additionally, several phenotypes of the leaf including an increased number of palisade mesophyll layers and an increased leaf thickness suggest that the era 1-2 plant is behaving as if it is under drought stress.

207 Expression of the Arabidopsis expansin gene AtExp7 is correlated with root hair formation

Cho, Hyung-Taeg and Cosgrove, Daniel J.

Department of Biology, Pennsylvania State University, 208 Mueller Laboratory, University Park, Pennsylvania, 16802

Root hair formation requires a localized cell expansion. Expansins are likely to function for cell enlargement via their unique wall-loosening capability. An Arabidopsis expansin gene AtExp7 is expressed in root hair cells. In this study we demonstrate the correlation between expression of AtExp7 and formation of root hairs in diverse hormonal, developmental, and environmental conditions. To show the gene expression pattern, AtEXP7::GUS was introduced into Arabidopsis plants using the Agrobacterium system. AtExp7 is expressed along the trichoblast cell files and root hairs in the wild type. The gene starts to express in a cell before the root hair initial appears. An ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), induces ectopic expression of AtExp7 and root hair initiation, whereas aminoethoxyvinylglycine, an inhibitor of ethylene synthesis, greatly decreased AtExp7 expression and root hair formation. Mutations at the TTG1 or GL2 loci caused AtExp7 gene expression and root hair formation in atrichoblast cells. A root hair-defective mutant, rhd6, lacks AtExp7 expression in the trichoblast cells. However, auxin, ACC and physical separation of the root from the agar plate induced both AtExp7 expression and root hair formation in rhd6 roots. These results show a

consistency between the expression pattern of AtExp7 and root hair formation, and suggest a probable role of expansins during localized cell expansion or differentiation.

208 Aux/IAA proteins are phosphorylated by phytochrome in vitro

Adan Colon-Carmona¹, Donna L. Chen, and Steffen Abel

University of California, Davis; ¹Current Address: University of Massachusetts, Boston

Aux/IAA genes encode short-lived transcription factors that are induced as a primary response to the plant growth hormone, indole-3-acetic acid (IAA or auxin). Gain-of-function mutations in several Arabidopsis genes, which alter invariant amino acids in conserved domain II of the encoded Aux/IAA proteins, cause phenotypic aberrations consistent with enhanced auxin responses, likely by promoting protein stability (Worley et al. [2000] *Plant J.* 21:553-562). Furthermore, the missense mutations in domain II of SHY2/IAA3 and AXR3/IAA17 induce ectopic light responses in dark-grown seedlings. Since genetic studies conducted by others suggest that the semi-dominant shy2 mutation bypasses phytochrome requirement for certain aspects of photomorphogenesis (Tian and Reed [1999] *Development* 126:711-721; Kim et al. [1998] *Plant J.* 15:61-68), we tested whether SHY2/IAA3 and other Aux/IAA proteins interact directly with phytochrome and whether they are substrates for its protein kinase activity. Here, we show that several recombinant Aux/IAA proteins interact with and are substrates for recombinant oat phytochrome A in vitro. Deletion analysis indicates that phytochrome A phosphorylation of Aux/IAA proteins occurs on the N-terminal half encompassing conserved domains I and II. We are currently mapping phosphorylation sites in IAA3. We have raised polyclonal antibodies against recombinant IAA3 to monitor levels of SHY2/IAA3 and phytochrome-dependent phosphorylation in vivo. We propose that phosphorylation of Aux/IAA proteins by phytochrome provides one molecular mechanism for integrating auxin and light signaling in plant development.

209 Molecular Genetic Analysis of Cytokinin Signal Transduction

I. D'Agostino, J. Deruere, G. Haberer and J. J. Kieber

University of North Carolina, Biology Department, Chapel Hill, NC 27599

Using differential display, we identified a cytokinin-responsive gene from Arabidopsis called IBC6 (induced by cytokinin). The IBC6 gene is a member of a large family of genes (ARR genes) in Arabidopsis, each of which contain a domain similar in sequence to the receiver domain of bacterial two-component response regulators. These ARR genes can be grouped into two distinct classes based on their sequence similarity, domain structure and their response to cytokinin. One class, the type A genes, display elevated transcripts levels within 10 min. of cytokinin application, and this induction is at least partially due to increased transcription. We have examined the pattern of IBC6 gene expression using fusions to reporter constructs and in situ hybridization, and we have begun to examine the function of these ARR genes in vivo. Results from these studies will be presented. We have also taken a genetic approach to identify cytokinin-signaling elements.

210 Studies on null alleles of RGA and GAI, repressors of gibberellin signaling in Arabidopsis

Alyssa Dill and Tai-ping Sun

Department of Botany/DCMB Group; Duke University; Durham, NC USA
RGA and GAI are negative regulators of gibberellin (GA) signal transduction in Arabidopsis. Because these genes are highly homologous, and single knockouts at these loci are phenotypically similar to wild-type Ler, it is likely that they are at least partially redundant in function. We initiated a detailed characterization of the null alleles *rga-24* and *gai-t6* to test whether these mutations have an additive effect on plant growth. Previously, *rga-24* was shown to partially rescue defects in *ga1-3*, a GA biosynthetic mutant with very low levels of bioactive GAs. These include abaxial trichome initiation, rosette radius, flowering time, stem elongation and apical dominance. We found that only the rosette radius and flowering time defects of *ga1-3* are partially restored in *gai-t6*, but to a much lesser degree than in *rga-24*. However, the combination of *rga-24* and *gai-t6* in *ga1-3* rescues all of the traits partially restored by *rga-24/ga1-3* to a wild-type or GA overdose phenotype. These results indicate that RGA and GAI have overlapping roles in GA signaling and that RGA plays a more dominant role in repressing the above traits. The trigenic mutant did not restore the flower development defect of *ga1-3*, suggesting that there is a third RGA/GAI homolog likely to be involved in flower development.

211 Genetic interactions among a network of transcription factors regulating seed development and ABA response

Finkelstein, R.R., Soderman, E., Brocard, I., and Lynch, T.J.

Department of Molecular, Cellular and Developmental Biology, UCSB, Santa Barbara, CA 93106, USA

The Arabidopsis abscisic acid (ABA)-insensitive *abi3-1*, *abi4-1*, and *abi5-1* mutants all have pleiotropic defects in seed development including slightly decreased sensitivity to ABA inhibition of germination and altered expression of some seed-specific genes. In addition, all three classes of mutants have some minor defects in vegetative ABA response. Although more severe alleles of *ABI3* result in a "green seeded" desiccation-intolerant highly ABA-insensitive phenotype, more severe alleles of *ABI4* and *ABI5* result in only partial resistance to ABA and do not impair acquisition of desiccation tolerance. These results suggest that loss of *ABI4* and *ABI5* function may be partially compensated by genetic redundancy. The *ABI3*, *ABI4*, and *ABI5* genes have all been cloned and found to encode transcription factors of the B3 domain, AP2 domain and bZIP factor classes, respectively (1-3). We have examined genetic interactions among these transcription factors by comparing phenotypes of mutants, ectopic expression lines, mutants carrying the ectopically expressed transgenes, and the corresponding wild-type lines. We have found evidence of cross-regulation of expression among these loci by RNA gel blot analyses and by assaying expression of promoter-GUS fusions in a variety of genetic backgrounds. These results suggest that these transcription factors function in a combinatorial network, rather than a regulatory hierarchy, controlling seed development and ABA response. Two-hybrid assays testing for direct physical interactions among these proteins are in progress.

1. Giraudat et al. (1992) *Plant Cell* 4:1251-1261
2. Finkelstein et al. (1998) *Plant Cell* 10:1043-1054
3. Finkelstein and Lynch (2000) *Plant Cell* 12: 599-610.

212 Molecular characterization of new members of the Arabidopsis PIN gene family
Jirí Friml^{1,2}, Justyna Wisniewska^{1,3}, Eva Benková^{1,4}, Thorsten Hamman⁵, Klaus Nettekheim¹, Petra Tänzler¹, Klaus Palme¹

¹Max-Delbrück-Laboratorium in der Max-Planck-Gesellschaft, Carl-von-Linné-Weg 10, D-50829 Köln, Germany, ²Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic, ³Department of Morphogenesis and Plant Physiology, Institute of General and Molecular Biology, 87-100, Torun, Poland, ⁴Institute of Biophysics ASCR, Královopolská 135, 612 65 Brno, Czech Republic, ⁵ZMBP Developmental Genetics, University of Tübingen, Auf der Morgenstelle 1, 72076 Tübingen, Germany

Polar auxin transport plays an essential role in many plant processes such as apical dominance, vascular development and tropism. We have started to systematically identify proteins and genes involved in this process using biochemical and genetic strategies. The AtPIN1 gene has been isolated by transposon tagging. Its possible role in auxin efflux has been discussed. The AtPIN gene family consists of about 10 members. We will present the analysis of several members of this family, including AtPIN3 and AtPIN4. AtPIN3 maps on chromosome 1, consists of 6 exons and 5 introns and encodes a protein of 640 amino acids sharing 73% similarity to AtPIN1. AtPIN3 is expressed in various tissues including stems, roots and seedlings. AtPIN3 was detected in columella cells of roots, in hypocotyl and in young stems on the lateral side of endodermis cells. Several transposon insertion mutants in the AtPIN3 gene have been isolated by reverse genetic screening. These mutants were analyzed in detail and shown to be null mutations. The lateral localization of the AtPIN3 protein, its high homology to AtPIN1 and the phenotype of the Atpin3 mutant suggest a role of the AtPIN3 gene in lateral auxin transport system. We also present a detailed analysis of the AtPIN4 gene. We will also show that auxin plays an important role in regulating lateral root initiation and adventitious root formation by describing recent progress. We will present detailed expression analysis of several AtPIN genes from the pericycle to the mature lateral root in wild type and mutant plants. These data support previous data on the crucial role of auxin transport in lateral root development and suggest a significant role of auxin efflux carriers in this process.

213 Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis

Majid Ghassimian^{1, 3}, Eiji Nambara¹, Sean Cutler^{1, 4}, Hiroshi Kawaide², Yuji Kamiya² and Peter McCourt¹

¹Dept. of Botany, University of Toronto, Canada. ²RIKEN, Wako-shi, Japan. ³Current address: Dept. of Biology and Center for Molecular Genetics, UCSD. ⁴Current address: CIW, Stanford, CA

Although abscisic acid (ABA) is involved in a variety of plant growth and developmental processes, few genes have been identified that actually regulate the transduction of the ABA signal into a cellular response. In an attempt to identify negative regulators of ABA signaling we identified two new alleles of the ETHYLENE INSENSITIVE 2 gene. Subsequent analysis indicates that other mutations that alter ethylene response also affect seed ABA sensitivity suggesting ethylene is a negative regulator of ABA signaling during germination. By contrast, at the level of root growth, a functional ethylene response pathway is required to positively regulate some aspects of ABA action. Surprisingly

ABA does not require ethylene to signal through this pathway, however, ethylene inhibits the use of this pathway by ABA. We discuss the response of plants to ethylene and ABA in the context of how these two hormones could influence a single pathway required for growth and development.

M. Ghasemian and E. Nambara contributed equally to this work and are listed alphabetically.

214 Isolation and characterization of a loss-of-function ERS1 (Ethylene response sensor1) mutant

1Hall, AE 2Young, J 1 Bleecker, AB

1University of Wisconsin-Madison 2Western Washington University

Ethylene perception in Arabidopsis is mediated by a family of five receptors that show homology to two-component histidine kinases. These genes have been divided into two subclasses based on sequence homology and overall structure. ETR1 and ERS1 belong to subclass I, and possess all of the consensus sequences predicted to be necessary for kinase activity, while subclass II genes (ETR2, EIN4, and ERS2) contain divergent kinase domains and contain an additional hydrophobic subdomain in the N-terminus of the protein. Our work has focused on defining the biochemical properties of ETR1 and ERS1. These proteins share biochemical similarities in that they both bind ethylene, form disulfide-linked dimers (Hall et al., in press), and interact with the raf-like kinase CTR1 in two-hybrid and in vitro assays (Clark et al., 1998) However, an important difference between these two proteins is that ERS1 lacks the C-terminal response regulator domain found in ETR1, which may have implications on downstream signaling.

Studies with Arabidopsis lines containing loss-of-function alleles of ETR1, ETR2, EIN4 and ERS2 in multiple combinations, confirmed that these genes are functionally redundant. In addition, the constitutive ethylene-response phenotype exhibited by plants lacking three or more receptors indicated that the ethylene receptors serve as negative regulators of the ethylene-response pathway (Hua and Meyerowitz, 1998). However, all of these studies were carried out in the presence of a functional copy of the ERS1 gene. In order to clarify the specific role of ERS1 in ethylene sensing we isolated a loss-of-function allele of ERS1 by screening T-DNA tagged populations. We have crossed the *ers1-2* mutant to loss-of-function mutants in the other ethylene receptor genes, and are characterizing the ethylene responses of plants containing multiple mutant combinations. Our analyses are focused on the seedling response to ethylene and will include studies exploring the kinetics of seedling response and recovery from ethylene exposure. These analyses will aid in defining the unique contributions of each receptor or subclass of receptors in ethylene perception, and will help answer our broader question of why Arabidopsis has five ethylene receptor genes.

215 Do two Arabidopsis O-GlcNAc transferase proteins function in a partially redundant manner in gibberellin signaling?

Lynn M. Hartweck, Tina M. Thornton, Neil E. Olszewski

Dept of Plant Biology, University of Minnesota

O-GlcNAc transferases (OGTs) modify proteins by transferring N-acetylglucosamine to specific serines and/or threonines. Biochemical studies of animal OGT proteins have

produced data consistent with the hypothesis that this modification regulates signal transduction cascades. Database searches indicate that OGT is a single copy gene in animals, but that there are two OGT-like sequences in plants. The first, the arabidopsis SPINDLY (SPY) gene was originally identified as a negative regulator of the gibberellin (GA) signal transduction pathway. This is consistent with the hypothesis OGTs regulate signal transduction. A second possible arabidopsis OGT, SPY2, has been identified by Genbank searching and has recently been cloned and sequenced. The hypothesis that SPY2 functions in a partially redundant manner to SPY is being tested. Recently, two T-DNA insertional alleles of SPY2 (*spy2-1*, *spy2-2*) have been identified and homozygous plants are being selected. A comparison of the phenotypes of the *spy2* mutants versus wild type and *spy* mutants will be presented. *spy2* mutants will be examined for the loss of O-GlcNAc protein modifications. By characterizing the degree of functional similarity between these two related proteins, more will be learned about the functioning of SPY2, SPY and the role of OGTs in signal transduction.

216 Sterols as Signaling Molecules in Plant Development Revealed by the *fk-J79* Mutant and the Expression Pattern of FACKEL

Jun-Xian He, Shozo Fujioka*, Shin Gene Kang, Huating Wang, and Jyan-Chyun Jang
Dept. of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210
*The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-0198, Japan

Although the profound effects of brassinosteroid hormones (BRs) on plant growth and development have been fully recognized, the functions of sterols other than BRs remain to be elusive. The plant C-14 sterol reductase is involved in the biosynthesis of both BRs and a variety of other sterols because it catalyzes the reaction that is upstream of the divergence of BR- and sterol-specific biosynthetic pathways. We cloned the FACKEL (FK) gene that encodes the C-14 sterol reductase via the analysis of a novel dwarf mutant *fk-J79*. The analyses of sterol compositions in *fk-J79* mutant reveal a reduction of both BRs and downstream sterols and an accumulation of both the precursor and 3 novel 8,14-diene sterols. However, unlike other known BR-deficient mutants, the hypocotyl elongation of neither *fk-J79* nor its allele *fk-X224* can be restored by exogenous BRs. This result indicates the importance of sterols other than BRs in development. To investigate the regulatory roles of sterols in development, we examine the effects of the 3 novel sterols and an inhibitor of C-14 sterol reductase in the seedling development. A phenocopy of *fk-J79* mutant can be obtained by treatments with the inhibitor, consistent with the notion that sterols are effective regulators of development. Furthermore, the expression of FK is strictly regulated by developmental stage and tissue type. It is highly expressed in the actively growing regions such as root tips, young leaves, anthers, ovules, and vasculature, suggesting its role in cell division and expansion. Since auxin- and BR-inducible TCH4 gene is highly expressed in *fk-J79* mutant, we further examine the influence of exogenous BRs and auxin on the FK expression. Our data demonstrate that the expression of FK is feedback regulated by brassinolide. Interestingly, its expression is enhanced by auxin, suggesting a complex regulatory circuit that controls the balance of hormone function.

217 The homeobox gene *ATHB5* affects abscisic acid mediated regulation of seed germination

Henrik Johannesson, Yan Wang, Johannes Hanson, Peter Engstrom

Dept. of Physiological Botany, Evolutionary Biology Centre, Villav. 6, 752 36 Uppsala, Homeodomain-leucine zipper (HDZip) proteins constitute a large family of transcription factors apparently unique to plants. Proteins of this class differ structurally from other classes of homeodomain transcription factors in that they contain a leucine zipper domain immediately C-terminal to the homeodomain. This structural feature suggests that these proteins bind DNA as dimers. The HDZip protein *ATHB5* interact with a 9 bp pseudopalindromic DNA-sequence, CAATNATTG, composed of two half-sites overlapping at a central position. Consistent with this finding we demonstrate that *ATHB5* forms a homodimeric complex in solution. Furthermore, *ATHB5* selectively heterodimerise in vitro with different HDZip proteins, suggesting that HDZip proteins constitute a complex network of homo- and heterodimeric transcription factors in plants. Histochemical staining of an *ATHB5* promoter-GUS fusion construct in *Arabidopsis* show expression localised specifically to the hypocotyl of germinating seedlings. Expression is strongest at the transition zone between the hypocotyl and the root and gradually declines along the hypocotyl axis. In order to assign a physiological role for *ATHB5*, we have generated transgenic lines harbouring *ATHB5* gene constructs controlled by the 35S CaMV promoter. These lines exhibit altered sensitivity to inhibition of germination and seedling root growth by the plant hormone abscisic acid. Our data suggest that *ATHB5* encodes a transcription factor active early after the onset of germination that take part in the abscisic acid mediated regulation of seedling development.

218 *CKH1*, a negative regulator of cytokinin signaling in *Arabidopsis*

Minoru Kubo¹, Yao-Guang Liu², Daisuke Shibata², Tatsuo Kakimoto¹

¹Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 5600043, Japan, ²Mitsui Plant Biotechnology Research Institute, Tsukuba, Ibaraki 3050047, Japan

Although cytokinins play an important role in plant development, the molecular actions of cytokinins in plants are not known well. To better understand the cytokinin signaling, we have screened for cytokinin-hypersensitive (*ckh*) mutants of *Arabidopsis* with elevated cytokinin sensitivity in a tissue culture system. The explants of *ckh* mutants induced rapidly growing calli with a green color at low levels of cytokinins, which are insufficient to induce such cytokinin responses in wild-type explants. All *ckh* mutants are recessive and fall into two complementation groups, *ckh1* and *ckh2*. Cytokinin levels of *ckh* mutants compared with wild-type were not elevated. We speculate that the *CKH* genes function as negative regulators of cytokinin signaling in *Arabidopsis*. The *CKH1* gene was mapped to the upper part of chromosome one and closely linked to a CAPS marker, m59. For map-based cloning of the *CKH1* gene, a contig that extended to 300kb were constructed with TAC (transformation-competent artificial chromosome) and BAC genomic clones, and one of the TAC clones complemented the *ckh1* phenotype. A gene carried by the TAC clone bore nonsense mutations in three *ckh1* alleles. We obtained the corresponding cDNA, and the functional analyses of *CKH1* are underway.

219 Microarray based analysis of auxin responsive genes

Marta Laskowski*, Reeta Prusty, Jian Hua, Paula Grisafi, and Gerry Fink
Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02142; * Williams College,
Williamstown, MA 01267

We are using cDNA microarrays to examine genes involved in auxin response and gravitropism in one snapshot. At present, the auxin response pathways remain largely unknown. Microarrays provide a relatively rapid assay that allows the discovery of genes involved in the response to auxin because they survey a substantial fraction of the genome at one time; the ensuing computational analysis permits the connection between metabolic pathways that is not possible by traditional mutant analysis. Using the non-redundant EST library that the stock center has made available, we have amplified 12,000 cDNAs, and have used a robotic printing device to create an Arabidopsis microarray. Preliminary results indicate that hybridizing this type of array with RNA from control and auxin-treated plants reflects anticipated differences in gene expression. Additional data will be presented at the meeting.

220 IAR1, a novel gene involved in auxin homeostasis

Jamie Lasswell, Luise E. Rogg, David C. Nelson, and Bonnie Bartel
Rice University, 6100 S. Main Street, Houston, TX 77005

The auxins are an important class of plant hormones involved in many aspects of plant development. The most common naturally occurring auxin is indole-3-acetic acid, or IAA. In Arabidopsis, up to 95% of the IAA pool is found conjugated to small molecules such as sugars and amino acids, and these IAA conjugates likely play an important role in regulation of IAA levels within the plant. However, the genes and enzymes involved in IAA conjugate metabolism are not yet well understood. We are investigating this process by isolating Arabidopsis mutants that respond abnormally to IAA-amino acid conjugates. We identified a mutant, *iar1*, that is resistant to the inhibitory effects of multiple IAA-amino acid conjugates on root elongation but that remains sensitive to free IAA. We used positional information to clone the IAR1 gene and found it to encode a protein with numerous transmembrane domains and several histidine-rich regions. The IAR1 protein has homologs in other multicellular organisms, including *Drosophila*, *C. elegans*, and mammals, and is similar in molecular structure to the ZIP family of metal transporters from Arabidopsis and yeast. Several models for IAR1 function in auxin homeostasis will be presented.

221 Arabidopsis 35S-cDNA lines for rapid identification of transcripts that cause phenotypes when overexpressed

Sherry LeClere and Bonnie Bartel
Rice University, 6100 S. Main, Houston, TX 77005

A system has been developed to overexpress random cDNAs in Arabidopsis thaliana following Agrobacterium tumefaciens-mediated transformation. We constructed an overexpression vector containing an Arabidopsis cDNA library driven by the Cauliflower Mosaic Virus (CaMV) 35S promoter. The vector, 35SpBARN, offers in terra selection with glufosinate ammonium (BASTA) herbicide and the ability to quickly identify the cDNA insert using flanking primers. We introduced this overexpression library into Arabidopsis and selected over 20,000 T1 transformants. We isolated 28 putative mutants

by screening T1 plants for individuals with visible morphological deformities. One of these mutants, V5, has a pale green pigmentation phenotype and overexpresses a partial *petH* cDNA encoding the C-terminus of chloroplast ferredoxin-NADP reductase, which co-suppresses the endogenous transcript. This mutant segregates in a 3:1 ratio in T2 progeny, consistent with a dominant mutation. Introducing a construct expressing either full-length or truncated *petH* cDNAs from the CaMV 35S promoter into wild-type *Arabidopsis* recapitulates the mutant phenotype, indicating that this system is overexpressing cDNA inserts to cause dominant phenotypes as expected. This system should yield phenotypes due to overexpression as well as co-suppression, making it a useful tool for identifying genes of interest. We are using this system to identify genes that confer auxin resistance when over- or ectopically expressed.

222 HYL1, a dsRNA-binding protein, integrates several plant hormone signaling pathways

Cheng Lu, Ryuji Tsugeki*, Nina V. Fedoroff

Plant Physiology Program and Biotechnology Institute, The Pennsylvania State University, University Park, PA 16802, USA * Present address: Department of Botany, Graduate School of Science, Kyoto University, Japan

We have identified a Ds insertion mutant of *Arabidopsis* designated *hyl1* (hyponastic leaves1) which displays abnormal responses to several plant hormones. The *hyl1* mutant has pleiotropic defects in seed development, including greatly increased sensitivity to ABA inhibition of germination and increased dormancy level. Roots of *hyl1* mutant seedling are hypersensitive to growth arrest by ABA. Two ABA-inducible genes exhibit significantly elevated basal levels of expression in *hyl1* mutant homozygotes. These results suggest that the HYL1 protein is a negative regulator of ABA responses. Other phenotypes of the *hyl1* mutant, such as a reduced root gravitropic response, an increase in the number of shoot branches, and a decrease in the number of lateral roots, suggest that the auxin response is abnormal. Analysis of the *hyl1* mutant's response to exogenous auxin revealed that the mutation reduces the plant's sensitivity to growth inhibition by auxin. The *hyl1* mutant also shows a reduced sensitivity to cytokinin in both dark and light conditions. We have cloned the HYL1 gene through using a TAIL-PCR (Thermal Asymmetric Interlaced PCR)-based strategy. Consistent with the pleiotropic phenotype of the mutant, HYL1 is ubiquitously and constitutively expressed in roots, leaves, stems, and flowers. The HYL1 gene is down-regulated by ABA, an observation that is consistent with the interpretation that HYL1 is a negative regulator in ABA signaling. The HYL1 gene encodes a polypeptide of 419 amino acids with two putative dsRNA binding motifs, six consecutive perfect repeats and a nuclear localization motif. Bombardment experiments using onion epidermal cells confirmed that the HYL1 protein localizes to the nucleus, suggesting a potential role in controlling gene expression. The HYL1 protein binds dsRNA, but not dsDNA, ssDNA and ssRNA. We postulate that HYL1 is a key regulator which integrates ABA, auxin and cytokinin signals.

223 Genetic studies of auxin homeostasis: IAA conjugate resistance and auxin supersensitivity

Mónica Magidin, Andrew Woodward & Bonnie Bartel
Rice University, Houston TX

Through their regulation of gene expression and cell growth, auxins affect many aspects of plant growth and development. It is therefore essential for plants to be able to regulate the amount of active auxin present in a given tissue at a given time. One mechanism through which this homeostasis is achieved is by conjugating indole-3-acetic acid (IAA, the most common natural form of auxin) to amino acids or sugars. In fact, more than 90% of IAA in *Arabidopsis* is found in conjugated forms. These conjugates are inactive, but can be hydrolyzed to release the free hormone. To better understand the functions and regulation of IAA conjugates, a variety of mutants that are resistant to IAA-amino acid conjugates but retain their sensitivity to free IAA were isolated (Bartel & Fink, 1995; *Science* 268:1745-1748). The *ilr2* mutant was isolated in this screen as IAA-leucine resistant. Our mapping data indicates that *ILR2* is a novel gene involved in IAA-conjugate metabolism that is localized within a 100 kb region on chromosome 3, between the markers *nga162* and *GL1*.

Auxin resistant mutants have provided valuable information about how auxin is sensed and regulated; they also suggest that a considerable part of auxin homeostasis is dependent on negative regulation. Hence, a second variety of auxin mutants could be isolated: auxin supersensitive (*axs*) mutants that would be defective in negative regulation of IAA homeostasis and/or signal transduction. Towards this end, we developed a modification of the root bending assay described by Howden & Cobbett (1992. *Plant Phys.* 99: 100-107) which allowed us to identify 11 *axs* mutants. These mutants are supersensitive to exogenous IAA in root elongation and/or in other auxin-mediated physiological processes.

224 Studies With a Viviparous Mutant of *Arabidopsis thaliana*, *spr2*

Javed I. Mirza, Glenn A. Galau and Russell L. Malmberg

Botany Department, University of Georgia, Athens, GA 30602, USA

Previously we reported a recessive spermine-resistant mutant of *Arabidopsis thaliana*, *spr2*, that was viviparous under ambient conditions of the growth room (Mirza & Rehman, 1998). Now we have studied the viviparous character of the mutant at different growth conditions. The mutant showed consistent vivipary at low to high humidity and varying temperatures. We have also observed that seed development of mutant plants is arrested at an early stage. The viviparous character is primarily embryonic although the influence of maternal genotype is not ruled out. We are also looking at ways to differentiate between the mutant and normal seeds in a segregating progeny. Preliminary studies using the phenotypic markers have suggested a linkage of *spr2* with chromosome 1.

225 Hormone-Resistant and Polyamine Metabolism Mutants of *Arabidopsis thaliana*

Javed I. Mirza, Cong Li, Wei Yu, Kimberly K. Emory, Russell L. Malmberg

Botany Department, University of Georgia, Athen, GA 30602, USA

A number of developmental mutants have been isolated from an EMS-mutagenized M2 population of *Arabidopsis* by screens for decreased sensitivity to spermine, NAA or BA. They include 15 NAA-resistant, 3 BA-resistant and 14 spermine-resistant mutants. The developmental abnormalities affect all growth stages from seed germination to seed formation, including seed development, altered seed shape, transparent testa, vivipary, affected root and/or hypocotyls gravitropism, absence or abundance of root hair, short

root hair, long hypocotyls, 1-3 cotyledons, dwarf or semi-dwarf stature, spirally-twisted growth of whole shoot, variation in leaf shape/size, twisting of rosette and cauline leaves, absence of trichomes, increased number of leaves, inflorescences and lateral branches, reduced apical dominance, malformed flowers, variation in the number and size of floral organs, homeotic conversions of floral organs, male or female sterility, reduced number of stigmatic hair, bifid or sunken stigma, crinkled or club-shaped to globose siliques, and pendulant or horizontal siliques. The phenotypes of the mutants are controlled by single recessive nuclear mutations. Some of the mutants are allelic to existing ones; others appear to be unique. Polyamines are synthesized by arginine decarboxylase (ADC) or ornithine decarboxylase (ODC). In Arabidopsis two genes encode ADC activity - ADC1 and ADC2. Previously we reported the isolation of recessive mutants, *spe1-1* and *spe2-1*, that reduce the levels of ADC activity in Arabidopsis. We have isolated another semi-dominant mutant with high ADC activity, *spe3-1D*. We generated gene specific probes for ADC1 and ADC2 mRNAs, and monoclonal antibodies specific for these two proteins. These tools let us examine ADC1 and ADC2 mRNA and protein levels in the *spe1-1* and *spe3-1D* genetic backgrounds. Both of the mutants had normal mRNA levels of ADC1 and ADC2, but had reduced protein levels in the *spe1-1* mutant and increased protein levels of ADC1 and ADC2 in the *spe3-1D* mutant. Genetic mapping studies show that neither *spe1-1* nor *spe3-1D* is linked to either ADC1 or ADC2 structural gene loci. The mutants *spe1-1* and *spe3-1D* regulate ADC enzyme activity from both structural genes at a post-transcriptional level.

226 Purification and cloning of NPA-binding plasma membrane aminopeptidases Angus Murphy and Lincoln Taiz

MCD Biology, University of California, Santa Cruz, CA 95064

Naphthylphthalamic acid (NPA)-binding proteins are putative negative regulators of polar auxin transport. We previously showed that NPA-amidohydrolases co-localize with flavonoid-sensitive plasma membrane (PM) Tyr-, Pro-, and Trp- aminopeptidases (APs) in Arabidopsis seedlings (Murphy and Taiz 1999). Moreover, artificial AP substrates and inhibitors blocked auxin efflux and low affinity specific NPA binding in a saturable manner (Murphy, et al. 2000). Thus the APs may play a role in the regulation of auxin transport. To further characterize the APs and associated proteins, NPA-binding peptidases with Tyr-AP activity isolated from Arabidopsis microsomes by gel-permeation, anion-exchange, and NPA-affinity chromatography were separated by SDS PAGE. Amino acid sequences of gel bands from a low-affinity fraction with Tyr-AP activity and a high affinity fraction with neutral endopeptidase activity were used to clone the corresponding genes from cDNA and genomic DNA libraries. From the weak-binding fraction, three glycoproteins with homology to mammalian PM metallopeptidases involved in processing of signaling peptides were identified. Four ER chaperonin proteins and three putative surface proteins co-purified with the AP-containing fraction. AtAP-M1 encodes a 100 kDa protein predicted to be a Type II transmembrane protein with its catalytic site on the external carboxy terminus. AtAP-M1 has the five domain signature of a puromycin-sensitive microsomal AP and shares a 75% homology with murine microsomal AP-A, an actin binding transmembrane peptidase. AtAP-P1 encodes an 82 kDa protein and is predicted to be an unanchored peripheral membrane AP-P type X-Pro peptidase (that cleaves X-Pro and Tyr N-terminal residues. AtNMP1 encodes a 56 kDa

protein and is predicted to be a Type Ib transmembrane neutral zinc metallopeptidase with its catalytic site on the cytoplasmic surface. Southern analysis indicated that all three are single-copy genes. The high affinity NPA-binding fraction contained two ~100 kDa glycoproteins and the 120 kDa p-glycoprotein AtPGP1. The ~100 kDa proteins are homologs of the putative receptor kinase CLV1 with an additional consensus C-term NMP catalytic site.

Murphy and Taiz (1999) *Plant Physiol. Biochem.* 37:413-443
Murphy, et al. (2000) *Planta* 210:In press

227 The role of flavonoids in Arabidopsis auxin transport, growth, and development
Wendy Ann Peer, Angus Murphy, Dior Kelley, Lincoln Taiz
MCD Biology, University of California, Santa Cruz, CA 95064
Flavonoids contribute variously to herbivore defense, UV attenuation, fertility, and seed dormancy (Debeaujon, et al 2000), and have also been implicated in the regulation of auxin transport and growth (Jacobs and Rubery 1988; Bernasconi 1996). In Arabidopsis seedlings, exogenous flavonoids displace the auxin transport inhibitor naphthylphthalamic acid (NPA) from plasma membrane (PM) vesicles and inhibit both NPA hydrolysis and the PM aminopeptidases (APs) that appear to hydrolyze NPA (Murphy and Taiz 1999; Murphy, et al. 2000). Evidence that flavonoids also regulate auxin accumulation in vivo was obtained using the flavonoid-deficient mutant *tt4*. In whole-seedling [¹⁴C] IAA transport studies of *tt4*, the wildtype (WT) pattern of auxin accumulation was altered. The defect appeared to be in auxin accumulation, as considerable amounts of auxin escaped from the roots. Treatment of the *tt4* mutant with the intermediate naringenin restored normal auxin distribution and accumulation in the root (Murphy, et al. 2000). Histochemical studies described here co-localize flavonoids with AP and NPA staining in a tissue and cell specific manner. Aglycone forms localize to the endomembrane system and PM, with some polar staining evident. A role in auxin transport regulation is further suggested by flavonoid localization in the vascular bundles of seedlings and inflorescence stems. In *tt* mutants deficient in flavonoid biosynthetic enzymes, intermediates accumulate at the same locations and developmental stages as WT (Ler) end products. Growth effects are evident as well. When compared to Ler, *tt4* and *tt5* seedlings have longer lateral roots and *tt4* has both altered location of roots and more roots overall. In 80 μE m⁻² s⁻¹ light, *tt3* and *tt4* flower earlier than Ler; high light intensity offsets this in *tt3* and produces shorter stems in *tt3* and *tt4*. Aglycone flavonoids, which are most effective in modulating auxin transport, appear to be restricted to growing tissues.

Bernasconi (1996) *Physiol. Plant.* 96: 205-510
Debeaujon, et al (2000) *Plant Physiol.* 122:403-424
Jacobs and Rubery (1988) *Science* 241: 346-349
Murphy and Taiz (1999) *Plant Physiol. Biochem.* 37: 413-443
Murphy, et al. (2000) *Planta* 210:In press.

228 Isolation of Arabidopsis mutants affected in stomatal regulation

Anna Chiara Mustilli, Sylvain Merlot, Bernard Genty*, Helen North+, Bruno Sotta°, Alain Vavasseur# and Jerome Giraudat

ISV-CNRS, Gif-sur-Yvette, France; *CNRS-Université Paris Sud, Orsay, France;

+INRA-Versailles, France; °UPMC, Paris, France; #CEA, Cadarache, France

Guard cell regulation permits the optimization of leaf gas exchanges (H₂O, CO₂) by integrating a variety of endogenous and exogenous signals, and is one of the most attractive plant systems to study signal transduction in response to different environmental stimuli. Second messengers, such as cytosolic free Ca²⁺ and cytoplasmic pH, with implication of protein kinases and phosphatases, have been previously reported in guard cell signaling. However, genetic dissection of signal transduction pathways in guard cells has been rather limited, at least in part because of the difficulty in identifying a reliable phenotype for mutant screening. Conversely, leaf temperature can be used as an indicator to detect mutants with altered stomatal responses, since stomatal aperture results in a rapid cooling of the leaf surface. The leaf surface temperature can be measured continuously and non destructively using infrared video thermography, and this technique permitted the isolation of the barley mutant cool which displayed ABA-insensitive guard (Raskin, I., and Ladyman, J.A.R. 1988, *Planta* 173, 73-78). We defined experimental conditions under which infrared video thermography can be reliably used to identify individual Arabidopsis plants with altered stomatal responses. We then performed a genetic screen for mutants that display a reduced ability to close their stomata in response to controlled drought stress. Some of the mutants recovered are deficient in abscisic acid (ABA) accumulation, whereas others seem to be specifically affected in stomatal regulation. Further characterizations of these new mutants, including responsiveness to ABA light and CO₂ will be presented.

229 IAA17/AXR3: Biochemical Insight Into an Auxin Mutant Phenotype

Ouellet, F., Overvoorde, P.J., and Theologis., A.

Plant Gene Expression Center, 800 Buchanan, Albany, CA 94710

Auxin transcriptionally activates a set of early genes that are thought to mediate the cellular and developmental changes associated with this hormone. The Aux/IAA gene family represent one class of early auxin-responsive genes and is composed of at least 22 members in Arabidopsis. Biochemical, molecular, and genetic studies suggest that the Aux/IAA proteins play a central role in auxin signaling. The short-lived and nuclear-localized Aux/IAA proteins contain four domains (I, II, III, and IV). Domain III and IV are also present in the ARFs and it is domain III that is responsible for homo- and heterodimerization among the various members of the Aux/IAA and ARF proteins. Arabidopsis plants carrying a mutation in domain II (P88L) of the IAA17/AXR3 protein exhibit pleiotropic morphological phenotypes and display altered auxin responses. Interpretation of the genetic data has led to the hypothesis that mutations in domain II enhances the stability of IAA17/AXR3. To experimentally test this hypothesis, we have used pulse-chase labeling with ³⁵S-Met of wild-type and axr3-1 seedlings followed by immunoprecipitation of IAA17 to determine its half-life. The data show that the axr3-1 mutation stabilizes the IAA17 protein by 7-fold. This finding supports the view that the dominant axr3-1 mutation is hypermorphic. Intragenic suppressors of the axr3-1 phenotype have been identified and contain second site mutations in domain I (axr3-1R3) or domain III (axr3-1R2). We have examined whether these second-site mutations

counteract the increased level of IAA17 by altering its nuclear localization or by affecting the capacity of the stabilized protein to form homo- or hetero-dimers. Results from transient expression assays of wild-type, mutant, and revertant forms of IAA17 fused to β -glucuronidase show that nuclear targeting is not affected. However, the revertant mutation in domain I (axr3-1R3) or domain III (axr3-1R2) affects the ability of the IAA17 to homo-dimerize. In contrast, only the mutation in domain III affects hetero-dimerization with IAA3/SHY2 or the ARF proteins. These results support the view that the homo- and hetero-dimerization of the Aux/IAA and ARF proteins are required for proper auxin signaling.

230 The RHA1 gene of *Arabidopsis thaliana* encodes a novel heat shock factor, possibly a transducer of auxin and gravity signals in root cells
S. Piconese, G. Tronelli, S. Ferrari, and F. Migliaccio
Institute of Plant Biochemistry and Ecophysiology; Consiglio Nazionale delle Ricerche; Monterotondo (Rome) Italy

We already reported the isolation of a new *Arabidopsis thaliana* root gravitropism and auxin physiology mutant. This mutant was named *rha1* for the additional characteristic of showing, when grown on a vertically oriented hard-agar plate, reduced or inverted right-handed slanting in the roots. The mutation is recessive and segregating through a Mendelian mechanism. The gene is tagged by a T-DNA insert. Taking advantage of the tag, through TAIL-PCR techniques, we cloned a fragment of the RHA1 gene close to the T-DNA left-border, and found, by comparison with already produced sequences, that it is inserted into the promoter region of a putative gene encoding for a new heat shock factor (HSF). This gene maps on chromosome 5, close and above the RFLP marker mi61. RHA1 shares a high degree of homology with HSFs from plants (*Arabidopsis*, tomato, maize, soybean), yeasts, *Caenorhabditis*, *Drosophila*, mouse and humans. RT-PCR is presently carried on to investigate if the message for the RHA1 protein is present in the wild-type but lacking in the mutant. A positive outcome of these experiment would strongly support the identity of RHA1 with the HSF gene carrying the T-DNA. We consider particularly significant the fact that RHA1 belongs to the HSFs. Recently, not only functions in the activation of the HSPs have been ascribed to these factors, but also in some critic cell and organ differentiation processes. We hypothesize that RHA1 could be part of the signal transduction pathways initiated by auxin and gravity. These signals should control gravitropism and the symmetry determination of the roots. A further support to our hypothesis is furnished by a recent research, through which it was shown that a HSF from humans regulates the activity of the A subunit of the PP2A phosphatase (Hong and Sargent 1999, *J.Biol.Chem.*,274:12967-70) . This phosphatase was in fact recently connected with the regulation of auxin transport in the *Arabidopsis* mutant *rcn1* (Garbers et al. 1996, *EMBO J.*15:2115-24). Possibly, we stumbled on a new piece of the signal transduction pathway that controls root tropic movements in plants.

231 Hsp90-buffered variation in *Arabidopsis*
Christine Queitsch¹, Andrew Evans², and Susan Lindquist^{1,3}
¹MGCB, The University of Chicago; ² The College of the University of Chicago;
³Howard Hughes Medical Institute, University of Chicago

In the fruit fly *Drosophila melanogaster*, the chaperone HSP90 buffers the expression of genetic variation affecting a wide variety of morphogenetic traits and releases it in response to environmental stress, suggesting that HSP90 may act as a capacitor for evolutionary changes.

By inhibiting HSP90 with the drug geldanamycin in developing *Arabidopsis* seedlings, we found that virtually all aspects of plants development and responses to environmental stimuli were affected. These were due to uncovering of genetic variation rather than a general destabilization of development because different traits were observed in different recombinant inbred lines and different ecotypes. Several phenotypes resemble well-known mutants in signal transduction pathways identified previously.

We are currently investigating the genetic architecture of these traits and their relationship to environmental stress.

232 PHENOTYPIC CHARACTERIZATION AND MAPPING OF HARLEQUIN (hlq) AND SHORT BLUE ROOT (sbr), TWO MUTANTS THAT ECTOPICALLY EXPRESS AN ABA-INDUCIBLE PROMOTER AND HAVE PLEIOTROPIC EFFECTS ON MORPHOGENESIS

Balasubramanian Rajagopal, Senthil Subramanian, Thomas Altmann*, Chris Rock
Dept. Biology, Hong Kong Univ. Sci. & Technol., *Max Planck Inst. Molec. Plant
Physiol., Gölm, Germany

Abscisic acid controls developmental processes as well as adaptation of vegetative tissues to various environmental stresses. In transgenic *Arabidopsis*, a carrot Late Embryogenesis Abundant (LEA) gene promoter, Dc3, drives tissue-specific expression in guard cells and root vasculature of the reporter gene GUS in response to ABA and drought (Chak et al. [2000] *Planta* 210: 875). We have isolated and mapped two dwarf mutants, harlequin (hlq) and short blue root (sbr), that ectopically express the Dc3-GUS reporter. HLQ maps 12.9 cM south of nga172 on chromosome III (LOD= 29.7), and SBR maps 3.6 cM from nga392 (LOD 6.6) on chromosome I. The sbr mutants are seedling lethal and have stunted roots that express Dc3-GUS at a higher than normal level. Seedlings heterozygous for sbr lack lateral roots and have roots intermediate in length between the parental type and mutants. IAA-conjugate levels are normal in sbr mutants. While the sbr mutant responds to GA, its phenotype is not rescued by exogenous GA or auxin. The hlq mutant was isolated based on the checkered pattern of Dc3-GUS expression in the epidermal layer of the roots and hypocotyl. The dwarf phenotype of hlq plant is not rescued by the application of GA, IAA, or CK. It has sparse root hairs and leaf trichomes. The flowers are sterile. Although the hlq mutant was isolated in the ABA-insensitive-2 (abi2) mutant background, the abi2 locus is not necessary for the hlq phenotype. Gene expression profiling of 15,000 *Arabidopsis* cDNAs with total RNA from hlq mutants showed significant up-regulation and down-regulation of a few genes. The analysis of ectopic gene expression and root shape mutants may help elucidate the molecular basis of tissue-specific and developmental regulation of morphogenesis in response to environmental and hormonal cues.

233 Subcellular Localization of the Ethylene Receptor ETR1

Melynda D. Randlett, Jennifer L. Findell, Xue-Chu Zhao, and G. Eric Schaller
Department of Biochemistry, Univ. of New Hampshire, Durham, NH 03824

The ethylene receptor ETR1 of Arabidopsis is a modular protein with ethylene-binding, histidine kinase, and receiver domains. In the amino terminal half of ETR1 is a hydrophobic domain containing three putative transmembrane segments; this domain is responsible for both ethylene binding and membrane localization. Sequence analysis does not provide information as to which membrane system of Arabidopsis the ETR1 ethylene receptor is localized. We have examined the membrane localization of ETR1 using several independent approaches. We fractionated Arabidopsis membranes by density-gradient centrifugation and by two-phase partitioning. The distribution of ETR1 after fractionation was compared to that of membrane markers. In addition, we generated green fluorescent protein (GFP) reporter constructs for both wildtype ETR1 (ETR1-GFP) and the ethylene insensitive mutant *etr1-1* (*etr1-1*-GFP). These were transformed into Arabidopsis and localized in living cells by fluorescence. Our results indicate that the ethylene receptor ETR1 localizes to the endoplasmic reticulum. The implications of this localization for ethylene signaling will be addressed.

234 A gain-of-function mutation in IAA28 suppresses lateral root development and alters auxin responses

Luise E. Rogg, Jamie Lasswell, Bonnie Bartel
Rice University, Houston, TX

Auxins are an important class of plant hormones, implicated in most aspects of plant development, and thus influence the overall size and shape of a plant. While the signal transduction pathways that sense and respond to auxin remain mysterious, a number of genes undergo dramatic transcriptional alterations in response to auxin. For example, the Aux/IAA genes were originally isolated based on strong and rapid transcriptional up-regulation induced by auxin. Aux/IAA genes are primary response genes whose products are thought to regulate auxin-responsive transcription. We cloned a new member of the Aux/IAA gene family, IAA28, based on the abnormal auxin responses and unusual auxin-related adult phenotypes of a gain-of-function mutant, including decreased apical dominance and extremely reduced lateral root formation. Northern analysis and promoter-GUS fusions demonstrate that IAA28 is strongly expressed in roots. IAA28 transcription is up-regulated in response to cycloheximide, suggesting it is a primary response gene. However, IAA28 transcriptional responses to auxin differ from all other characterized members of the Aux/IAA gene family. Experiments with the auxin-inducible BA-GUS construct suggest that IAA28 is a repressor of auxin-induced transcription. Future experiments will illuminate the role that IAA28 plays in auxin responses, particularly lateral root formation.

235 Dissecting the early response of the Arabidopsis ATL2 gene to potential elicitors

Mario Serrano, Jaime Bravo, Raul Salinas, Laura Aguilar and Plinio Guzman
Genetic Engineering Department. Cinvestav-IPN, Unidad Irapuato. Irapuato, Gto, Mexico

ATL2 is a member of a multigene family coding highly related RING-H2 zinc finger proteins. Analysis of the expression of ATL2 indicates that a rapid increase in ATL2 mRNA accumulation occurs after incubation with chitin or with cellulases. This accumulation is transient, declining after 30 minutes of incubation. An increase in GUS activity is also observed in Arabidopsis plants carrying the promoter fused to GUS after

15 minutes of incubation with such elicitors, Arabidopsis plants transformed with a fusion of pATL2-GUS show a localized pattern in the leaf primordia of young seedlings and incubation with cellulase triggers expression through all the seedling. Ethylene may also participate on ATL2 expression since inhibition of ethylene biosynthesis or ethylene response induces its expression. We are exploiting the pattern of expression of a pATL2-GUS fusion to screen for mutants that modify this pattern. We are selecting for plants that show constitutive expression of the GUS fusion and plants where expression of GUS is not triggered upon incubations with elicitors. We expect that such screening uncover components of the early response to elicitors. Early-response genes play central roles in signal transduction pathways, often modulating secondary responses. These genes frequently encode very unstable transcripts and after stimulus perception the time preceding a response is generally short. The 3'-untranslated region (3'UTR) of ATL2 revealed sequences which appear to be key determinants for rapid transcript decay, suggesting that it encodes an unstable transcript. We found that a fragment of the 3'UTR of ATL2 indeed decrease transcript accumulation to a reporter construct in transgenic Arabidopsis plants; we are currently dissecting the 3'UTR of ATL2 to unravel possible determinants for transcript decay. This work is supported by a grant from CONACyT, México.

236 The eir1 Suppressor Screen: a Novel Tool to study Auxin Signalling
Harald F. Sigmund, Tobias Sieberer, Marie-Theres Hauser and Christian Luschnig
Center of Applied Genetics, University of Agricultural Sciences, Muthgasse 18, A-1190
Vienna, EU

Despite the broad knowledge about auxin effects in higher plants, still very little is known about the underlying pathways on a molecular basis. The recent cloning of the putative root-specific auxin-efflux carrier EIR1 (Ethylene Insensitive Root 1) enabled the initiation of a novel genetic screen in Arabidopsis thaliana. The screen is based on the altered root phenotype of eir1 mutants and its modification due to changes in expression of genes involved in auxin metabolism and auxin signalling. For this purpose, eir1-3 mutant plants containing the GUS reporter gene, fused to the auxin-inducible PIG4-promoter, were transformed with the activation tagging vector pSKI15 (a kind gift from Detlef Weigel, Salk Institute), to create gain-of function as well as loss-of function mutants. From about 14000 primary transformants, T2 seed pools were collected and screened for suppression of the mutant phenotype. Approximately 70000 individual plants were examined, and more than 500 candidates exhibited a significant alteration of the eir1 phenotype. T3 plants with a confirmed phenotype were examined in a secondary screen for their PIG4::GUS activity. This allowed us to discriminate between auxin-response and unrelated phenotypes. To date, about 30 candidates with a significantly altered reporter gene expression pattern were isolated, which will be subjected to molecular and functional analysis. Details of the screen as well as preliminary data from selected candidates will be presented.

This work is supported by grants 13353-GEN and P13948-GEN from the FWF

237 High level expression of ATHB7 in transgenic Arabidopsis causes a suppression of elongation growth consistent with a role of ATHB7 in the drought stress response

Eva Soderman, Mattias Hjellstrom and Peter Engstrom
University of Uppsala, Evolutionary Biology Center, Dept. of Physiological Botany,
Villav.6, S752 36 Uppsala, Sweden

The *Arabidopsis thaliana* homeodomain-leucine zipper encoding gene *ATHB7* is induced by water deficit conditions in an abscisic acid (ABA) dependent manner. We have investigated the role of *ATHB7* by use of transgenic *Arabidopsis* with altered levels of expression of *ATHB7* as the result of the activity of a constitutively expressed *ATHB7* gene, or of a suppression of *ATHB7* expression due to the expression of an antisense *ATHB7* mRNA. Transgenic plants, over-expressing *ATHB7*, show a phenotype with reduced elongation growth, increased branching and early flowering under short day conditions. Transgenic plants expressing an antisense *ATHB7* mRNA did not differ from wild-type in development. A translational fusion of the *ATHB7* promoter with the reporter gene *GUS* (*ATHB7::GUS*) in transgenic *Arabidopsis* plants showed the promoter activity from the *ATHB7* gene to be highly dependent on drought stress. Strong *GUS* activity was detected in leaves and in root tips of primary and lateral roots when the transgenic seedlings were exposed to drought stress conditions. By contrast, only weak *GUS* expression was observed in seedlings grown under optimal growth water conditions.

238 A Role for Brassinosteroids in Germination

Camille M. Steber¹ and Peter McCourt²

¹USDA-ARS, Washington State University, 209 Johnson Hall, Pullman WA 99164-6420 USA, csteber@wsu.edu. ²University of Toronto, Dept. of Botany, 25 Willcocks St., Toronto ON M5S 3B2 CANADA

The plant hormones abscisic acid (ABA) and gibberellins (GA) play antagonistic roles in controlling seed dormancy and germination. ABA promotes dormancy and inhibits germination, while GAs break dormancy and promotes germination. Indeed, strong GA biosynthetic mutants fail to germinate unless rescued by application of exogenous GA. This poster presents evidence that brassinosteroids (BR) also stimulate germination. Physiological and mutation analyses have demonstrated that BRs are required for cell elongation, flowering, epinasty, and skotomorphogenesis. However, no BR mutant has been shown to have a germination phenotype. Our first clue that BR may play a role in seed germination came from the observation that BRs rescue the germination of both GA biosynthetic mutants and of GA-insensitive *sleepy1* mutants. It may be the case that this rescue of germination is due to stimulation of cell elongation by BRs increasing the pressure on the seed coat from within. However, auxins are also known to stimulate cell elongation, but do not stimulate germination (Koornneef And Karssen 1994). This may mean that BR does have a role in germination. To test this hypothesis we examined the response of *bri1* seed to increasing doses of ABA. *bri1* mutant seed show an increased response to ABA in germination. This suggests that sensitivity to BR is needed for normal germination potential. If GA and BR act to stimulate germination, this raises the question of whether they do so by dependent or parallel pathways. The fact that BR stimulates germination of a GA-insensitive mutant indicates that it does not act by stimulating GA biosynthesis directly. However, it is possible that there is "crosstalk" between the two pathways. For example, GA mutants may have increased BR sensitivity

or visa versa. This seems plausible since the two hormones act on many of the same aspects of plant development including cell elongation and fertility.

Koornneef, M. and C.M. Karssen 1994. "Seed Dormancy and Germination" in *Arabidopsis*. E.M. Meyerowitz and C.R. Somerville eds, pp. 313-334

239 The molecular interaction between NPH4/ARF7 and MSG2/IAA19 in the yeast two-hybrid system

Kiyoshi Tatematsu¹, Emmanuel Liscum² and Kotaro T. Yamamoto¹

¹Graduate School of Environmental Earth Science, Hokkaido University, Sapporo, 060-0810 Japan, and ²Division of Biological Sciences, University of Missouri, Columbia, MI 65211 USA

In order to understand the role of auxin in differential growth, we have carried out screening of *Arabidopsis* mutants that do not exhibit growth curvature of hypocotyls upon unilateral application of auxin-containing lanolin paste to hypocotyls. We obtained a recessive and a dominant mutant, *msg1* (Watahiki and Yamamoto 1997) and *msg2*, respectively. Both mutants exhibited auxin-resistance in hypocotyls, as well as defects in phototropism, gravitropism, and apical hook maintenance. *msg1* has been shown to be allelic to *nph4* (Liscum and Briggs 1995, Stowe-Evans et al. 1998) and *tir5* (Ruegger et al. 1997) mutations. NPH4/TIR5/MSG1 has been shown to encode an auxin-response factor (ARF) 7 (Harper et al. 2000), while MSG2 has been shown to encode an Aux/IAA protein, IAA19 (Tatematsu et al. 10th Intl. Conf. Arabidopsis Res. 1999). ARF and Aux/IAA proteins share a carboxy-terminal domain (CTD), and can form homo/heterodimers through CTD interactions (Kim et al. 1997, Guilfoyle et al. 1998). Since phenotypes of *msg2* are similar to those of *nph4*, products of the mutated MSG2/IAA19 gene may form a heterodimer with NPH4/ARF7 in the *msg2* mutant, resulting in interference of normal function of ARF7. In order to test this possibility, we have studied molecular interaction between ARF7 and IAA19 proteins using the yeast two-hybrid system. IAA19 and the CTD of ARF7 were fused to the Gal4 activation domain (AD) and Gal4 DNA binding domain (BD), respectively. For comparison, AD fusion of IAA1 and IAA6, and BD fusion of ARF8 CTD were also checked. The qualitative two-hybrid assay showed that CTD of ARF7 interacted with IAA19, IAA6 and IAA1 to a similar extent. The mutation in IAA19 that corresponds to *msg2-1* did not affect the heterotypic interaction significantly. The ARF8 CTD also interacted with the three IAA proteins. These results suggest that ARF7 CTD can interact with many IAA proteins. We are now performing quantitative analyses of the molecular interaction between ARF7 and IAA proteins in an attempt to sort out potential relevant interactions.

240 Characterization of SPY and the role of an O-GlcNAc transferase in plant hormone signaling

Tina M. Thornton and Neil E. Olszewski

Dept. of Plant Biology, University of Minnesota, St. Paul MN 55108-1095 USA

Gibberellins (GAs) are involved in most aspects of plant growth and development including germination, elongation growth, flowering, and fruit development. Although the biosynthesis of this tetracyclic diterpene carboxylic acid hormone has been well characterized, relatively little is known about the mechanism of GA action. SPINDLY

(SPY) has been identified as a negative regulator of GA signal transduction. All of the phenotypes of GA deficiency are at least partially suppressed by spy mutations. SPY shows significant sequence similarity with animal O-GlcNAc transferases (OGTs). Based on this similarity, it is hypothesized that SPY modifies cytosolic and nuclear proteins by the addition of O-linked N-acetylglucosamine (O-GlcNAc). This suggests that post-translational modification of proteins by the addition of O-GlcNAc plays a crucial role in plant development. The effect of GA treatment on the pattern of O-GlcNAc protein modification will be presented and compared to the pattern of O-GlcNAc modification found in spy mutants. Data from the functional analysis of the SPY protein will also be presented.

241 Functional studies of the TPR domain of SPY

Tong-Seung Tseng(1), Steve Swain(2), Neil Olszewski(1)

(1) Department of Plant Biology, University of Minnesota, MN 55108; (2) CSIRO, Australia

Gibberellin (GA) is a plant hormone that regulates many aspects of plant growth and development. Little is known about how plants respond to GA. The SPINDLY (SPY) protein has been shown to negatively regulate the GA signal transduction pathway of Arabidopsis. Recessive spy mutations cause phenotypes consistent with increased GA signaling, including early flowering and germination on paclobutrazol, an inhibitor of GA biosynthesis. SPY contains 10 tetratricopeptide repeats (TPR) at the N-terminus and exhibits similarity to the C-terminus of O-GlcNAc transferase (OGT) from *C. elegans*, human and rat, suggesting the involvement of O-GlcNAc protein modification in GA signaling. The TPR repeats are found in a variety of proteins, including animal OGT, and are known to mediate protein-protein interactions. Therefore, it is possible that SPY functions by interacting with other proteins through TPR-mediated protein-protein interactions. To gain further insight into the importance of functional domains of SPY, we sequenced fifteen new spy alleles. The results show that both TPR domain and the OGT domain are important in SPY's proper functioning in GA signal transduction. The phenotypes of plants overexpressing the TPR domain mimic those of spy mutant, further indicating that the TPRs of SPY are important. The TPR domain was also used in the yeast two hybrid screening to look for SPY-interacting proteins from Arabidopsis.

242 The Arabidopsis impotent1 Mutant Is Defective in the Allene Oxide Synthase (AOS) Gene for Jasmonic Acid Biosynthesis

Chunxin Wang, Seth Glatstein, Xiaozhong Bao, and Zhongchi Liu

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20740

The impotent1 (*imp1*) mutant was isolated by screening T-DNA insertional lines. No visible phenotypic defect was observed except that the mutant flowers are male sterile. Close examination revealed that stamen filaments of *imp1* flowers are shorter and the locules of the mutant anthers were unable to dehisce. A single T-DNA insertion into the AOS (allene oxide synthase) gene was responsible for the mutant phenotype. Since the insertion occurred upstream of heme-binding domain of the AOS protein, *imp1* is likely a null loss-of-function mutant. Northern analysis indicated that the transcription of AOS gene was precociously terminated in the mutant. As AOS is the first enzyme in the

biosynthetic pathway of jasmonic acid (JA), *imp1* mutant must be unable to synthesize JA. Thus, exogenous application of JA might be able to rescue the mutant phenotype. We found that *imp1* mutant was indeed able to set seeds after exogenous application of JA. However, similar exogenous application of linolenic acid (the substrate of AOS) failed to rescue the male sterile phenotype of the *imp1* mutant. In addition, the *imp1* mutant also showed high susceptibility to a fungal pathogen *Pythium irregulare*, which could be rescued by JA application as well. Taken together, our results demonstrated that JA plays an important role in pollen maturation and plant defense, which has been suggested by other mutants affecting either JA signal pathway (*coi1*) or JA biosynthesis (*fad3-2 fad7-2 fad-8* triple mutant). The susceptibility of *imp1* to bacterial and insect pathogens and the expression of defensin gene PDF1.2 in *imp1* are being investigated.

243 Is BRI1 the brassinosteroid receptor

Wang, Z-Y1., Vafeados, D1., Cheong, H1., Redfern, J1., Friedrichsen, D1., Yin, Y1., Hanson, K1., Asami, T2., Yoshida, S2., and Chory, J.1

1Howard Hughes Medical Institute, Plant Biology Laboratory, The Salk Institute, La Jolla, CA USA 92037. 2 The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

Brassinosteroids (BRs) are a class of steroid hormone involved in multiple plant developmental processes. BR biosynthetic and insensitive mutants such as *det2* and *bri1*, respectively, show many characteristics of light-grown plants when grown in the dark and are dwarfs when grown in light. Molecular genetic studies have led to the identification of several genes involved in BR biosynthesis and only one gene, *BRI1*, required for BR responsiveness. *BRI1* is a membrane bound receptor kinase with a leucine-rich-repeat (LRR) extracellular domain, a transmembrane domain, and a cytoplasmic kinase domain with serine/threonine specificity. A popular model to be tested is that *BRI1* is the BR receptor. We have studied the function of *BRI1* in BR signal transduction. Transformation of *Arabidopsis* with a *BRI1*-GFP fusion construct showed that *BRI1* is expressed mostly in young tissues that undergo cell expansion, and that the *BRI1*-GFP protein is localized to the plasma membrane. Transgenic plants overexpressing the *BRI1*-GFP fusion protein showed increased BR sensitivity and increased BR binding activity in their membrane fractions. A *bri1* mutant with reduced *BRI1* protein level has reduced BR binding activity. Furthermore, the anti-GFP antibodies immunoprecipitated the BR binding activity from the *BRI1*-GFP transgenic plants. These results indicate that *BRI1* is at least part of the BR receptor. The native *BRI1* protein behaves as a high molecular weight complex in gel filtration chromatography. The effects of BR treatment on the kinase activity and protein complex formation of *BRI1* are being investigated. To identify additional components for BR action, we screened for *det2* suppressor mutants and mutants insensitive to the BR inhibitor brassinazole (Brz) and isolated several new mutants related to BR responses. Of these, we have cloned two cytochrome P450s that, when overexpressed, suppress the *det2* phenotype and increase cell elongation of wild type plants. A mutant showing reduced sensitivity to Brz has been characterized and the corresponding gene is being identified by positional cloning.

244 Expression Analysis of Two-Component Regulators from *Arabidopsis*

Xue-Chu Zhao¹, Todd A. Richmond², Chris Somerville², and G. Eric Schaller¹

1Dept. of Biochemistry, Univ. of New Hampshire, Durham, NH 03824; 2Dept. of Plant Biology, Carnegie Institution of Washington, Stanford, CA 94305

Two-component regulators are involved in sensing and transmitting environmental stimuli. The two-component system makes use of a phospho-relay that incorporates histidine kinases, response regulators, and sometimes phosphotransfer proteins. In *Arabidopsis*, proteins with homology to all members of two-component regulators have been found. These are implicated in signal transduction by the hormones of ethylene and cytokinin, and in osmosensing. We examined expression levels of *Arabidopsis* two-component regulators using DNA-chip technology. Expression was analyzed following treatment of plants with ethylene, salt or light. In addition, we made use of mutant *Arabidopsis* plants altered in ethylene perception. Expression of some ethylene receptors was stimulated by ethylene. In addition, expression of ethylene receptors was found to decrease upon exposure of plants to salt stress. The implications of these results for ethylene signaling will be addressed.

245 Genetic Analysis of Indole-3-Butyric Acid Response Mutants

Bethany K. Zolman, Melanie Monroe-Augustus, Beth Thompson, and Bonnie Bartel
Rice University; 6100 S. Main, Houston, TX, 77005

Auxins are a family of plant hormones that influence numerous aspects of growth and development, including apical dominance, vascular development, tropic responses, inhibition of root elongation, and initiation of lateral roots. Indole-3-butyric acid (IBA) is an endogenous auxin that efficiently induces rooting and is consequently widely used in commercial and agricultural settings. Two hypotheses have been proposed regarding the mechanism of IBA action. IBA could act solely through its conversion to the more abundant auxin indole-3-acetic acid (IAA) in a pathway similar to fatty acid β -oxidation, which would occur in the peroxisomes. Alternatively, IBA itself could function as an auxin, either with its own signal transduction pathway or via the IAA pathway. To better understand the *in vivo* role of this naturally-occurring auxin, we have identified 14 *Arabidopsis* mutants that are resistant to the inhibitory effects of IBA on root elongation, but that remain sensitive to IAA. A subset of the mutants also have developmental defects in the absence of exogenous sucrose, suggesting that they may have defects in peroxisomal fatty acid chain shortening. Other mutants appear to have normal peroxisomal function; some of these may be defective in IBA transport, signaling, or responses. These mutants represent at least ten complementation groups, and the seven that we have mapped represent novel mutants in *Arabidopsis*. We have positionally cloned the genes defective in three of these mutants. Two encode proteins expected to function in peroxisome biogenesis, whereas the third encodes a protein hypothesized to function directly in β -oxidation. These results indicate that in *Arabidopsis*, IBA acts, at least in part, via its conversion to IAA.

246 KEULE, a Sec1 gene required for cytokinesis in *Arabidopsis*

Farhah Assaad^{1,4}, Yoann Huet², Ulli Mayer³, Chris Sommerville⁴ and Gerd Juergens³

1. Lehrstuhl fuer Genetik, University of Munich, 80638 Germany;

2. Pflanzenwissenschaften, ETH-Zurich, 8092 Switzerland; 3. Entwicklungsgenetik,

University of Tuebingen, Germany; 4. Carnegie Institute of Washington, DPB, Stanford,

CA 94305

KEULE is required for cytokinesis in *Arabidopsis thaliana*. We have positionally cloned the KEULE gene and show that it encodes a Sec1 protein. Co-suppression of the native KEULE gene gives rise to cytokinesis-defective mutant sectors in all the somatic tissues of wild-type transformants. As Sec1 proteins are known to bind syntaxins, we have tested for an interaction between KEULE and the cytokinesis-specific syntaxin KNOLLE. We show that KEULE and KNOLLE interact. This protein-protein interaction would recruit the otherwise soluble KEULE protein to membranes, and in fractionation experiments KEULE is indeed membrane associated in dividing but not quiescent tissues. KEULE's cycling on and off membranes is being studied with the help of GFP fusions. Consistent with the cytokinesis-specific keule phenotype, the KEULE protein is expressed predominantly in dividing tissues. Surveying the *Arabidopsis* genome has revealed two highly conserved homologues of KEULE, and these are being characterized. KEULE is the first Sec1 gene shown to be required for cytokinesis in any organism. The study of cytokinesis in both the plant and animal kingdoms has classically focussed on the cytoskeleton. It is only more recently that work on plants has highlighted the importance of membrane dynamics during cytokinesis. Cytokinesis in plants is achieved by targeting and fusing Golgi-derived vesicles at the equator of a dividing cell and, to this effect, both KEULE and KNOLLE are required for membrane fusion events. Sec1 proteins in other organisms have been demonstrated to have a large number of interacting partners, to integrate a large number of intra and/or intercellular signals, and to regulate vesicle trafficking with respect to these signals. As a Sec1 protein, KEULE will thus most likely represent a novel link between cell cycle progression and the membrane fusion apparatus.

247 Analysis of meristem activity and evaluation of polyploidization in *Arabidopsis* roots

Manfred Axterer, Elisabeth Bauer and Marie-Theres Hauser

Center of Applied Genetics, University of Agricultural Sciences Vienna, Muthgasse 18, AUSTRIA; hauser@mail.boku.ac.at

Cell division and cell expansion are the primary parameters of plant growth. Cell division in roots is restricted to the meristematic zone at the tip. In order to define the root meristem and to measure the onset of mitotic activity upon germination we used a transgenic marker line expressing a labile translational fusion between the mitotic cyclin B and the GUS reporter gene (kind gift from John Celenza). The first cells which start to divide upon germination are the uppermost epidermis cells adjacent to the root hypocotyl junction. Hence, the activation of the quiescent embryonic meristem is initiated from the basal end of the root. Once the root meristem is activated, it enlarges and the number of cells in mitosis increases. This enlargement is dependent upon growth conditions and a highly significant correlation exists between the size of the root meristem and the number of dividing cells. Thus, proliferation of roots is regulated by changing the size of the meristematic zone and as a result the number of dividing cells. Furthermore, this analyses allow to define the root meristem as the zone between the initials and the most distal cell before rapid cell elongation occurs. The zone where the root cells reach their final dimension is called cell expansion zone. In *Arabidopsis* roots this zone accommodates 6 to 10 tiers of cells and is about 500 μm long. As cell expansion is the second major parameter of root growth we evaluated cell sizes of different tissues and found that

atrachoblast are 1.2 times longer than cortex cells and that latter are 1.3 times longer than endodermis cells. As cell size is thought to correlate with ploidy levels we quantified the DNA content in different root tissues. Most of the cells in the cell expansion zone have a ploidy level of 4C. The nuclear DNA content increases to 8C in older differentiated parts of the root. Nuclei with 16C were only observed 5 mm distal of the quiescent center in epidermis and cortex but not in endodermis cells. These data shows that dividing cells of the root can switch to endoreduplication and this switch is not synchronized in adjacent cells.

This work funded by the EU grant PL 960217

248 Molecular analysis of the tonneau mutations

Juliette AZIMZADEH, Christine CAMILLERI, Anna CHRISTODOULIDOU, Philippe NACRY, Martine PASTUGLIA and David BOUCHEZ

Laboratoire de Biologie Cellulaire, INRA-Centre de Versailles, Route de Saint-Cyr, 78026 Versailles, France

Recessive mutations in the TON genes result in dwarf, misshapen plants whose cells fail to elongate properly and do not have oriented division planes. The overall morphology is strongly affected in those two classes of mutants (i.e. ton1 and ton2), yet all pattern elements are present in their correct relative positions. Defects in cell elongation and division seem to be connected to abnormalities in cortical cytoskeleton arrangement as observed by tubulin immunolabelling, that is a disorganized interphase microtubule cortical array and a lack of pre-prophase band before mitosis.

TON1a and TON1b are nearly identical genes organized in tandem repeat which are both inactivated in the ton1 mutant by a 1,4kb deletion. They encode 30 kDa acidic proteins with no yet characterized functional domains but significant similarities with proteins of other organisms including higher animals are detected. TON2 is a 55kDA polypeptide homologous to type 2A-protein phosphatases regulatory subunits, with a C-terminal domain related to calmodulin and centrin. We showed by complementation analysis that TON1 genes are functionally redundant. They are both expressed constitutively and at a low level, as TON2 is, in wild-type Arabidopsis plants.

In an attempt to localize the TON1 gene products, we raised polyclonal antibodies against these polypeptides. TON1 proteins associate with microsomal fractions in a pH-dependent manner and are found in plasma membrane-enriched fractions, where remaining cortical cytoskeleton elements are expected. Furthermore, they seem to co-localize with α -tubulin in cytoskeleton extracts from Arabidopsis cultured cells.

A two-hybrid screen has revealed an interaction between TON1b and centrin, an ubiquitous calcium-binding protein associated with microtubule organizing centers in some lower plants and yeast, whose function in higher plants is unknown however. In an in vitro binding assay, this centrin interacts with TON1b in a calcium-dependent manner. The relevance of such interaction in vivo , as well as a possible direct interaction between TON1 and TON2, the latter being partly homologous to centrin, is currently under study.

249 AtVPS45 complex formation at the trans-Golgi network

Diane C Bassham, Anton A Sanderfoot, Valentina Kovaleva, Haiyan Zheng and Natasha V Raikhel

DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312

Several different pathways for the transport of proteins to the yeast vacuole have been identified. Vps45p is a protein shown to be required for two of these pathways; (1) the well-studied pathway through the secretory system taken by Carboxypeptidase Y, which also requires the integral membrane t-SNARE Pep12p, and (2) an alternative route directly from the cytosol taken by Aminopeptidase I, requiring the t-SNARE Tlg2p. AtVPS45 is a Vps45p-like protein from Arabidopsis that can complement the yeast vps45 mutant phenotype. We now demonstrate that AtVPS45 is present in two different protein complexes at the trans-Golgi network (TGN). AtVPS45 interacts with two t-SNAREs, AtTLG2a and AtTLG2b, that show similarity to the yeast t-SNARE Tlg2p, but does not interact with AtPEP12. AtTLG2a and b each co-localize with AtVPS45 at the TGN; however, AtTLG2a is in a different region of the TGN to AtTLG2b by immunogold electron microscopy. We therefore propose that complexes containing AtVPS45 and either AtTLG2a or b define functional sub-domains of the TGN, and may be required for different trafficking events. An additional SNARE, AtVTI1b, is also found in each complex containing AtVPS45. When expressed in yeast, AtVTI1b can function in the Aminopeptidase I pathway, and not in the Carboxypeptidase Y pathway. Based on the data presented, one or both of the complexes containing AtVPS45 may be involved in a new transport pathway to the plant vacuole, equivalent to the yeast Aminopeptidase I pathway. Large-scale immunoprecipitation experiments are now underway to isolate other components of these complexes. The function of AtVPS45 in vivo is also being addressed by the isolation of T-DNA insertion mutants.

250 LRX1 is a new type of cell wall protein involved in root hair morphogenesis

Nicolas Baumberger and Beat Keller

University of Zürich, Switzerland

Extensins form a major group of structural cell wall glycoproteins. They are involved in the mechanical strengthening of the plant cell wall during development and in response to pathogen infection or wounding. They might also contribute to determine the structure and properties of the cell wall during cell differentiation. Here we report the molecular and functional characterization of LRX1, a new gene encoding an extensin protein containing a leucine-rich-repeat (LRR) domain. LRRs are known to mediate protein-protein interactions in a wide range of processes. The expression of LRX1 is restricted to the root and preferentially to differentiating trichoblasts. We introduced a c-myc tag in the LRX1 sequence and could identify on western blot the tagged LRX1 as a polypeptide of 180 kDa. Immunolocalization detected LRX1 specifically in developing and mature root hairs. No protein could be detected in the rest of the trichoblast cells. We identified three independent En-I tagged mutants of LRX1 which all show the same alteration in root hair morphogenesis. The *lrx1* mutants exhibit correct root hair initiation but subsequent development is disturbed. The root hair basis is often swollen, elongation stops early and root hairs frequently show branching. The *lrx1* mutant phenotype is phenocopied by constitutive expression of the LRR domain of LRX1, whereas constitutive expression of the complete LRX1 gene does not influence root hair development. A truncated LRX1 most likely interferes with the normal function of WT LRX1 by binding to the legitimate interacting partner of the LRR domain. The mutant

phenotype suggests that LRX1 is involved in the tip growth expansion process, which is characteristic of root hairs, pollen tube and fungal hypha. LRX1 might contribute to determine and maintain the very strict polarization of the elongating root hair.

251 Xyloglucan Endotransglycosylases of Arabidopsis: Diversity of Genes, Expression Regulation and Function

J. Becnel, P. Campbell, E. Iliev, D.H. Polisensky and J. Braam

Rice University, 6100 Main St, Houston TX 77005

Plant cells are surrounded by a cell wall that determines the shape and size of cells and of the plant itself. Previous work has shown that TCH4 of Arabidopsis encodes a xyloglucan endotransglycosylase (XET) that has the potential to modify xyloglucan polymers, important components of the cell wall. The TCH4 gene is strongly upregulated in expression by environmental stimuli, including touch, darkness, and temperature extremes. Upstream sequences of the TCH4 transcribed region confer appropriate regulation of expression to the luciferase reporter gene. Real time regulation of gene expression is monitored as light emissions from transgenic plants. Stimulated plants glow with defined spatial and temporal patterns.

XETs cleave xyloglucans and religate the newly generated ends to other xyloglucan polymers. Although this activity is well defined, the physiological consequences remain undetermined. Sequence homology has revealed an extensive gene family that encodes XET-related (XTR) proteins. The predicted proteins share between 37% and 85% identity as well as a conserved motif (DEIDFEFLGN) that likely contributes to the active site. We are investigating the functional significance of this gene family by characterization of primary protein structures, enzymatic activities, regulation of gene expression and consequences of loss of function.

252 Subcellular localisation of KORRIGAN, a membrane-bound endo- β -1,4-glucanase required for normal cellulose synthesis in Arabidopsis

Bichet, A., F. Goubet, B. Satiat-Jeunelemaître 1, M. McCann 2, S. Pagant. S. Vernhettes and H. Höfte

Laboratoire de Biologie Cellulaire, INRA, Route de Saint-Cyr, 78026 Versailles cedex. 1 Institut des Sciences Végétales, CNRS, Gif-sur-Yvette, France. 2 John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, United Kingdom

KORRIGAN encodes an endo- β -1,4-glucanase which is required for normal cell elongation and wall assembly. In contrast to other EGases which are secreted proteins, KOR is membrane-bound (Nicol et al., 1998). Here we show that cell walls of seedlings mutant for KOR are deficient in alkaline-resistant cellulose. A cellulose deficiency was also observed in hypocotyl cells using FTIR μ spectroscopy. These observations suggest that normal synthesis and/or crystallisation of cellulose requires the activity of this EGase. A similar role for an EGase was reported previously for *Agrobacterium tumefaciens* (Matthysse et al., 1995). As expected for a protein involved in the synthesis of cellulose, KOR levels are high in elongating cells and during secondary cell wall deposition. We will report on the subcellular localisation of the protein as shown by immunofluorescence in the wild type and in other cellulose-deficient mutants.

Nicol et al. 1998. EMBO J., 17, 5563-5576.

Matthysse et al., 1995. J Bacteriol, 177, 1076-1081.

253 The Arabidopsis CaaX Protease AtAFC1 Can Restore Yeast a Factor Secretion
Bracha, K., Eldar, H. and Yalovsky, S.

Department of Plant Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv 69978,
ISRAEL

Following prenylation proteins undergo two prenyl-dependent modifications at their carboxy-terminal end. The first of these modifications is the proteolytic removal of the three carboxy-terminal residues of the CaaX box prenylation-signaling motif. In *Saccharomyces cerevisiae*, CaaX box proteolysis is catalyzed by two ER/golgi-localized, and unrelated zinc-metalloproteases called AFC1 and RCE1. Although AFC1 has a higher affinity to the a factor, the activities of both enzymes overlap and a factor secretion is only inhibited in *afc1 rce1* double mutant cells. Here we report cloning and characterization of AtAFC1, an Arabidopsis homologue of yeast AFC1 protease. AtAFC1 was cloned by a PCR-based approach using oligonucleotide primers designed according to a sequence available from the Arabidopsis genome project. A 1.3 Kb fragment was amplified and the deduced amino acids sequence shows 54% identity to yeast and human AFC1. Interestingly, the length of AtAFC1 mRNA that was deduced by the genome project contains only 900 bases. Sequence analyses revealed that the additional 400 bases are internal and were misinterpreted as an intron region. Functional complementation of mutant yeast cells was demonstrated by pheromone diffusion halo assays. Growth inhibition halos of *sst2* type cells were not formed around *afc1 rce1* double mutant cells because these cells can no longer secrete the a factor. Halos were formed when AtAFC1 was over expressed in *afc1 rce1* cells from a 2⁺ high copy number plasmid, under the strong GPD promoter. Halos that were formed around AtAFC1 complemented or wild type cells were of comparable size. Thus, AtAFC1 is a true functional homologue of the yeast AFC1 protease. These data should now enable a full characterization of prenylation and related modification in plants and identification of the targeting pathways of prenylated proteins in plant cells.

254 Genetic placement of CPR5, a disease resistance gene, in the trichome development pathway

Ginger M. Brininstool and John C. Larkin

Louisiana State University

CPR5 is a gene involved in disease resistance which also has abnormal trichomes. Plants mutant for CPR5 constitutively express pathogen resistance genes, are dwarfed, have lesions on many of their rosette leaves, and experience early cotyledon senescence. In addition, the leaf trichomes on mutant *cpr5* plants are reduced in size and branch number, lack papillae, and have a "glassy" appearance. *cpr5* trichomes also have reduced endoreplication. In an effort to elucidate the function of CPR5 in trichome development, genetic and biochemical analyses have been done. Analysis of double mutants between two *cpr5* alleles and known trichome mutants, *gl1-1*, *gl2-1*, *gl2-p1*, *gl3-1*, *try-jc*, *sti*, and *zwi*, has allowed the placement of CPR5 in the trichome development pathway. CPR5 acts downstream of transcription factors involved in trichome initiation, upstream of a negative regulator of cell expansion, and upstream of a cytoskeletal protein involved in cell expansion. The glassiness of *cpr5* trichomes, prompted us to look at their

birefringence under polarized light. *cpr5* mutants have almost complete loss of birefringence. Birefringence has been correlated with organized cellulose in the cell wall. The cellulose content of *cpr5* leaves is significantly less than WT leaves. Preliminary results suggest that the non-cellulosic carbohydrate content of the cell walls of *cpr5* leaves is qualitatively the same as WT.

255 Identification of genes important for methylation of the SUPERMAN locus

Xiaofeng Cao, James P. Jackson and Steven E. Jacobsen

Dept. of MCD Biology, UCLA, Los Angeles, CA 90095-1606

Several epigenetic alleles of the Arabidopsis flower development gene SUPERMAN (SUP) have been identified which show dense cytosine methylation of the SUP DNA (the *supm* alleles) (Science 277, 1100-1103). SUP also becomes densely hypermethylated de novo in plants containing an antisense-MET1 transgene, or the recessive *ddm1* or *ddm2* mutations (Current Biology 10,179-186).

We are using two approaches to identify genes that are important for methylation of the SUP locus. First, we have identified a new class of putative cytosine methyltransferases in Arabidopsis (the DRM genes) that show homology to the animal Dnmt3 de novo DNA methyltransferases (PNAS, in press). We have isolated loss of function Arabidopsis T-DNA insertion mutations in each of three DRM family members and will present our characterization of the effects these have on SUP methylation, and on overall genome methylation.

Our second approach is a genetic screen for mutations that abolish the methylation at SUP and hence suppress the *supm* phenotype. We found 7 families that contain putative *supm* suppressor mutations, one of which maps near the DRM1 and DRM2 loci on chromosome V. We are currently testing whether this suppressor locus is identical to one of the DRM loci, and will present this data along with our analysis of the other suppressor mutants.

256 The Role of the Arabidopsis ELD1 Gene in Cell Development and Photomorphogenesis in Darkness

Jin-Chen Cheng, Kvin Lertpiriyapong, Susanna Wang, and Zinmay Renee Sung

Department of Plant and Microbial Biology, University of California, Berkeley CA 94720

Because cell growth and differentiation are regulated by complex interactions among different signaling pathways, a growth defect affects subsequent differentiation. We report on a growth-defective mutant of Arabidopsis called *eld1* (elongation defective). Cell elongation was impaired in every organ examined. Later characteristics of the *eld1* phenotype include defective vascular tissue differentiation, the inability to grow in soil, ectopic deposition of suberin around twisted vascular bundles, the de-etiolation phenotype, and continuation of shoot development and flowering in the dark. The dwarf phenotype of *eld1* could not be rescued by treatment with exogenous growth regulators. Because defective cell elongation is the earliest and most universal feature detected in *eld1* mutants, control of or activity in cell elongation may be the primary function of the ELD1 gene. The impaired cell growth results in pleiotropic effects on cell proliferation and differentiation, and the retardation in hypocotyl elongation enables growth and development in darkness.

257 Quantitative and qualitative control of mitotic cyclin levels in the regulation of organ growth during Arabidopsis development

Adán Colón-Carmona^{1, 2}, Hang Phung¹, Tal Haimovitch-Gal¹, Ratha You¹ and Peter Doerner^{1, 3}

¹Salk Institute for Biological Studies, La Jolla, Ca 92037; ²University of Massachusetts, Boston, MA 02125; ³University of Edinburgh, Edinburgh EH9 3JR, UK

The regulatory architecture of the Arabidopsis mitotic cyclin CycB1; 1 promoter is simple and consists of three interacting functional cis-elements. A negative element, a positive element, and a G2/M-phase specific cell cycle element mediate transcriptional control in both synchronized cell cultures and transgenic Arabidopsis. The cell cycle element is sufficient to direct cyclin expression in dividing cells. In the absence of the repressor element, the positive and the cell cycle-specific elements together direct higher than wild-type levels of cyclin mRNA to the root and shoot apical meristems, which results in enhanced growth rates of both root and shoot organs. In addition, plants over-expressing cyclin proceed through development quicker than wildtype, particularly under short day conditions. Thus, quantitative and qualitative elements in the promoter of CycB1; 1 act in concert to control cell division rates and organ growth.

258 Identification of a rapid subcellular response to wounding by imaging plants expressing random GFP::cDNA fusions

Sean R. Cutler, David W. Ehrhardt, Joel S. Griffitts, Chris R. Somerville
Carnegie Institution of Washington, Department of Plant Biology, 260 Panama Street, Stanford CA 94305

Many problems in plant cell biology can be informed by the direct observation of subcellular structures and their associated dynamics in living cells. To facilitate live-cell investigations, we have explored a high-throughput approach to examining subcellular organization by producing and imaging large numbers of plants that express random GFP::cDNA fusions. Fluorescence imaging of 5700 transgenic plants indicated that approximately 2% of lines expressed a fusion protein with a different subcellular distribution than that of soluble GFP. Our results suggest that a wide variety of subcellular structures and dynamic processes can be marked efficiently using random GFP::cDNA libraries.

The potential of this approach for identifying unique dynamic processes in living cells is demonstrated by the identification of a GFP fusion protein that displays a rapid subcellular redistribution after wounding. A fusion between GFP and the enzyme Nitrilase 1 was found to redistribute from the cytoplasm into granular structures minutes after delivery of a wound stimulus. Cells at a distance from the primary wound site display GFP::Nit1 granulation, suggesting that the fusion protein is responsive to a motile signal produced rapidly after mechanical damage to Arabidopsis cells.

The function Nitrilase performs in wound responses is being approached using biochemical, genetic and cell biological approaches. To help elucidate the composition and function of GFP::Nitrilase granules, a granule purification protocol is being developed. Genetic screens using the marker should be a powerful approach to identifying components of the signaling pathway and characterizing its biological role in vivo. Collectively, our observations suggest that the rapid formation of granules reveals a

novel wound-induced signal and response pathway. The ability to visualize this process in vivo using GFP::Nitrilase will facilitate our analysis of this pathway. Our results indicate that screening GFP::cDNA libraries is a useful approach for identifying and visualizing components of subcellular structures in higher plant cells, and that it is capable of revealing novel insights into subcellular dynamics.

259 Characterization of *Arabidopsis thaliana* Cdc48 in Cell-Plate Biogenesis

Carrie Dickey, Dave Rancour, and Sebastian Y. Bednarek

Department of Biochemistry, University of Wisconsin, Madison, WI 53706

Cell-plate assembly is required during plant cytokinesis to separate the cytosol and organelles into two daughter cells. Formation of the cell-plate becomes evident when secretory vesicles consolidate and fuse across the equatorial center of the cell. The cell-plate expands outward from the equatorial center and eventually joins with the plasma membrane as additional vesicles fuse. Little is known about the fusion events that underlie this process. The localization of the syntaxin homolog Knolle (KN) and an *Arabidopsis thaliana* homolog of the vesicle fusion protein Cdc48p (AtCdc48p) at the cell-plate suggests that an analogous N-ethylmaleimide sensitive factor (NSF)- and/or AtCdc48p-mediated mechanisms characterized in mammalian and yeast systems may be involved. To gain insight into the role of AtCdc48p during cell-plate assembly, we have generated antibodies against AtCdc48p. These antibodies were used to define the protein's membrane association and to demonstrate colocalization of AtCdc48p with KN at the cell-plate and other intracellular vesicles. In addition, we are using a complimentary reverse genetic approach to examine the function of AtCdc48p in vivo. Transgenic plants expressing antisense AtCDC48 cDNA and dominant negative forms of the protein have been generated. We have also identified an *Arabidopsis* AtCDC48::T-DNA insertional mutant which disrupts the AtCDC48 reading frame. Further characterization of these mutants will be essential to understanding the role of AtCdc48p in the process of cell-plate formation.

260 Functional Characterization of *Arabidopsis thaliana* " Extra-Large GTP-Binding Protein" (AtXLG1)

Lei Ding and Sarah M. Assmann

Department of Biology, The Pennsylvania State University, University Park, PA 16802, USA

Heterotrimeric GTP-binding proteins, which are composed of α , β , and γ subunits, are implicated in signal transduction responses of plants to stimuli such as blue light, pathogens and hormones. In our laboratory, the gene encoding a special GTP-binding protein, "extra-large GTP-binding protein", was isolated from *Arabidopsis thaliana* (Lee and Assmann, 1999). The carboxy-terminal region of AtXLG1 is homologous to a G β protein, while a portion of the amino-terminal region of AtXLG1 matches the consensus sequence of the bacterial TonB-box. To characterize the function of AtXLG1, we made both antisense and overexpression constructs driven by the 35S promoter. By analyzing the antisense and overexpression transformants, we hope to find clues that point to the function of AtXLG1. We are also making AtXLG1 promoter:GUS constructs to investigate AtXLG1 expression patterns in *Arabidopsis thaliana*. Additional work is also being conducted to test whether the protein has the characteristics of a G β protein. So far,

it has been demonstrated by GTP- γ -³⁵S labeling that AtXLG1 expressed in *E. coli* has GTP-binding activity (Lee and Assmann, 1999). However, the instability of the His-tagged AtXLG1 prohibited further purification of the expressed AtXLG1, which restricted our efforts to use purified AtXLG1 to test its GTP hydrolytic activity. Alternative expression systems are being explored. Progress in these approaches will be reported at the meeting. This research is supported by USDA grant 98-35304-6681 to S.M.A.

Lee, Y.R.J. and S.M. Assmann (1999) *Plant Mol. Biol.* 40: 55-64

261 Three *Arabidopsis* mutants with guard mother cell cytokinesis defects
Tanya Falbel¹, Lisa Koch¹, Jeanette Nadeau², Fred Sack², and Sebastian Y. Bednarek¹
¹Department of Biochemistry, University of Wisconsin, Madison WI ²Department of Plant Biology, Ohio State University, Columbus OH
Guard mother cells undergo a specific symmetric division to form the guard cells of stomata in leaves. Three *Arabidopsis* mutants affecting this specialized division were identified in a screen for guard cell distribution pattern or morphology defects. They identify three separate genetic loci and map to positions on chromosomes I, III, and V. All three mutants have partial or missing cell walls in their stomata, with each locus defective to a varying degree. The mutant with the most severe cytokinesis defect, J62B, is a semi-dwarf plant, shows subtle changes in leaf shape, is late flowering, and only produces fertile flowers and seed under certain growth conditions. Another mutant with almost as severe a cytokinesis phenotype, F100, is an extreme dwarf plant, expresses high levels of anthocyanins, and only after several months develops an inflorescence which never produces complete floral organs. The third mutant, *cyd1* (cytokinesis defective-1) has significant but less frequent cytokinesis defects than the other two mutants, and cytokinesis is defective in most cell types of the shoot. *cyd1* is viable as a homozygote, the plants are of normal size, yet the seed set is poor. This is likely due to a gametophyte defect, similar to that observed in another cytokinesis defective mutant, *tso1*. The map interval for each of these loci has been narrowed to several hundred kilobases. Based upon the observed phenotypes, candidate genes in each map interval are being tested for complementation. Additionally, cytoskeletal staining patterns in the guard cells of these mutants is being analyzed to determine whether the arrangement of the cortical arrays, spindle, or phragmoplast differs from wild type.

262 Isolation of three *Arabidopsis* genes encoding novel cytoskeletal proteins using a polyclonal antibody against bovine MAP2/Tau proteins

John Gardiner, Nimalika Weerakoon, John Harper and Jan Marc
University of Sydney, Australia

We have used a polyclonal antibody against bovine MAP2/Tau microtubule-associated proteins to identify related cytoskeletal proteins in plants. Immunoblotting of protein extracts from *Arabidopsis* seedlings showed several immunoreactive bands 60kDa-110kDa in size. In immunofluorescence microscopy of BY-2 tobacco and *Arabidopsis* cell cultures and wheat and onion root tip squashes, the antibody labelled several distinct cytoskeletal structures. In interphase cells it labelled vesicles associated with cortical actin and fine filaments about the nucleus, and the spindle pole region was labelled in

anaphase. We have isolated cDNAs for three different proteins by screening an Arabidopsis expression library with the polyclonal antibody. Clone 3AA is for a 110kDa kinesin-like protein showing a high homology (39% identity in 451aa) to centromere protein E, a known kinetochore motor protein (1,2). Clone 5CB encodes a 95kDa protein which again has similarity to centromere protein E (19% identity in 451aa) and also rabbit myosin (20% identity in 510aa) at its carboxy-terminus. At the amino terminus is a putative myristylation site, suggesting it may link the cytoskeleton to a membrane. Clone 12AC encodes a 114kDa protein with similarity (19% in 863aa) to the DCLIP-190 protein from Drosophila which binds microtubules and also associates with myosin (3). In addition all three clones show strong sequence homology to MAP2 and/or Tau proteins. The use of polyclonal antibodies to proteins from distantly related organisms for expression library screening appears to be an efficient way of isolating cDNAs of cytoskeletal proteins in Arabidopsis.

1. Yen, T.J., Li, G., Schaar, B.T., Szilak, I. and Cleveland, D.W. (1992). *Nature* 359 (6395), 536-539.
2. Yen, T.J., Compton, D.A., Wise, D., Zinkowski, R.P., Brinkley, B.R., Earnshaw, W.C. and Cleveland, D.W. (1991). *EMBO J.* 10 (5), 1245-1254.
3. Lantz, V.A. and Miller, K.G. (1998). *Journal of Cell Biology* 140(4), 897-910.

263 Mutants of vegetative actin isoforms display subtle phenotypes in roots
Laura U. Gilliland and R. B. Meagher
University of Georgia; Genetics Dept, Life Sciences Bldg; Athens, GA 30602-7223
Actin proteins are involved in numerous cytoskeletal processes affecting plant development and are encoded by large multigene families in higher plants. We hypothesize the ancient and divergent subclasses of plant actin genes have been preserved through evolution because they have unique patterns of expression and/or encode actin proteins with specialized cytoskeletal functions. We previously identified T-DNA insertion mutants in two of the three vegetative actin genes, ACT2 and ACT7, through sequence-based screening efforts and these alleles appear to be deleterious mutations in multigenerational population studies. Northern and western analysis reveals the act2-1 allele is essentially null and a bulbous root hair phenotype is directly attributable to the act2-1 mutation. Homozygous act7-1 mutants display wavy and twisted roots on vertical plants and produce seeds with reduced and delayed germination. The act7-1 mutants produce wild type levels of RNA but the level of actin protein is reduced nearly ten-fold. A double mutant for these two actin genes is developmentally arrested and displays profound phenotypes in both shoot and root development. Therefore, while enough redundancy exists in the actin gene family of Arabidopsis thaliana that single actin mutants have only mild morphological phenotypes, disruption of multiple actin genes with related expression patterns prevents the plant from performing vital tasks.

264 N-glycosylation and the plant cell wall: cloning and phenotypic analysis of knopf
Stewart Gillmor and Chris Somerville
Carnegie Institution of Washington and Stanford University 260 Panama St. Stanford CA 94305

In a genetic screen for mutations that affect cell expansion in the Arabidopsis embryo, we identified 8 new alleles of *knopf* (Mayer et al., 1991). Cells of *knopf* embryos primarily expand laterally, not longitudinally. As a result, late stage mutant embryos are nearly identical in size to wt, but are morphologically similar to a radially-swollen heart stage embryo.

knopf embryos accumulate high levels of callose in their cell walls, and transmission electron microscopy of bent-cotyledon stage embryos reveals that *knopf* cell walls are 20-25% the width of wt embryo walls. Cellulose levels in *knopf* embryos are approximately 5% of those in wt, suggesting an explanation for the thin-walled, radially swollen phenotype of *knopf* mutants.

We have identified the *KNOPF* gene by positional cloning. *KNOPF* is predicted to encode a protein with ~35% identity to yeast and mammalian glucosidase I (β -1,2 glucosidase), the first trimming enzyme in N-linked glycan processing. Interestingly, mutations in yeast glucosidase I cause a 50% reduction in cell wall β -1,6 glucans (Jiang et al., 1996; Romero et al., 1997). This effect is specific to the yeast cell wall, as the glucosidase I mutation does not affect the folding and export of glycoproteins that are not involved in cell wall biosynthesis (Simons et al., 1998).

Loss of *KNOPF* protein function may lower cellulose levels by affecting the folding and/or activity of glycoproteins involved in plant cell wall biosynthesis. Our immediate goals are to determine whether the *KNOPF* protein does indeed have β -1,2 glucosidase activity, localize the protein within the cell, and to try and identify specific glycosylated cell wall enzymes that are compromised in their folding or function in the *knopf* mutant background.

265 Genetic analysis of chloroplast biogenesis

Lucero Gutiérrez-Nava[•], Stewart Gillmor[§] and Patricia León[•]

[•]Institute of Biotechnology, UNAM, Cuernavaca, Morelos, México [§]Carnegie Institution of Washington, Stanford, CA

We are interested in genes involved in the early biogenesis of the chloroplast. To this end, we have screened through 1000 EMS M3 families, 5000 T-DNA families, and examined all chlorophyll pigmentation mutant lines available from the ABRC stock center. To focus exclusively on mutations which affect early chloroplast biogenesis, we have limited our analysis to lines that we designate as true albinos. We define albinos as mutant seedlings which totally lack normal green and yellow photosynthetic pigments. Of the 140 pigmentation lines recovered in our screen, we define 10 as true albinos. Thus, the number of nuclear genes in our study which mutate to an albino phenotype in Arabidopsis is approximately 7% of the total number of pale green and yellow seedling mutations.

Mapping and complementation analysis reveals that we have identified one new allele of the *cla1* gene (which is involved in early chloroplast development) as well as mutations at 6 novel loci which map to chromosomes III, IV and V. Previous analysis of our EMS families, using reference alleles, suggests that approximately 2000 M3 families represents mutagenesis of one genome equivalent. By these criteria, we estimate that we have recovered mutations in approximately half of the nuclear genes which mutate to an albino phenotype in Arabidopsis.

To classify our albino mutants using molecular markers of chloroplast development, we have analyzed the accumulation of transcripts of RbcS, CAB, and CLA1 in the above mutants. The results of this analysis suggest that all 6 mutant loci have a lesion very early during chloroplast development. In addition, we have classified our albino mutants as affecting cell autonomous and non-cell autonomous factors, by observing development of the mutants during embryogenesis. Mutants affected in a cell autonomous factor remain completely albino during late stages of embryogenesis, while the hypocotyls of non-cell autonomous mutants become progressively more green. This may be due to diffusion of a factor from the maternal plant to the embryo which allows chloroplast development to proceed at a slow rate. We are currently analyzing plastid biogenesis and leaf development in our albino mutants using electron and light microscopic analysis.

266 XPO1 from Arabidopsis mediates nuclear export of proteins containing leucine-rich nuclear export signals

Dorothea Haasen and Thomas Merkle

Universitaet Freiburg, Institut fuer Biologie II / Zellbiologie, Schaenzlestr.1, D-79104 Freiburg, Germany

The nuclear envelope provides a physical barrier that allows spacial separation of fundamental processes such as transcription and translation. Translocation across the nuclear envelope is mediated by nuclear transport receptors of the importin beta family. We identified Exportin1 (XPO1/CRM1) from Arabidopsis as the nuclear export receptor for proteins carrying leucine-rich nuclear export signals (NESs). XPO1 was functionally characterised by its interaction with NESs of animal and plant proteins and with the small GTPase Ran1. Nuclear export activities in vivo were demonstrated using an assay based on the localisation equilibrium of a GFP reporter protein containing both a nuclear localisation signal (NLS) and an NES motif. We investigated the localisation of the Arabidopsis protein RanBP1a and identified a leucine-rich NES at the carboxy terminus of the protein that was functionally indistinguishable from the Rev NES. Arabidopsis XPO1 is sensitive to the cytotoxin leptomycin B (LMB). As a consequence nuclear export of NES-containing proteins in vivo is inhibited by LMB. Our results demonstrate that the machinery for the nuclear export of proteins is functionally conserved among animals, yeasts and plants, and that active nuclear export may play an important role in the partitioning of regulatory proteins in plants.

267 Bonsai, a Phospholipid Binding Protein, Is Required for Growth at Low Temperature

Jian Hua, Paula Grisafi, and Gerald R. Fink

Whitehead Institute for Biomedical Research, Cambridge, MA 02142

Plants achieve their final size by regulating cell expansion and division in response to the environment. To uncover the mechanism for this response, we identified genes involved in the process by screening for mutants that have abnormal plant size. One such mutant, dubbed bonsai(bon), has tiny and curly leaves and short thin stems. The reduction in plant size is due to a reduction both in cell size and in cell number. However, these bon plants are fertile miniatures. Overexpression of the Bon gene under the control of the 35SCaMV promoter results in plants with larger leaves and thicker stems. Thus, Bon plays a critical role in controlling the size of the plant.

Northern blot analysis shows that the Bon gene is expressed mostly in leaves and stems. GUS reporter analysis indicates that it has a dynamic expression pattern--Bon is more strongly expressed in young growing cells, not in older ones. The bon phenotype is expressed in a temperature dependent manner. The bon mutant is indistinguishable from the wild type when grown at relatively high temperature, but is dwarf when grown at low temperature and humidity.

BON encodes a protein with sequence homology to a family of copine proteins found in human, worm, and paramecium. The N-terminus of the protein family has two C2 domains that can bind phospholipid in a calcium-dependent manner. The C-terminus of the protein family is a putative protein-protein interaction domain. It has been speculated that the copine proteins are involved in vesicle fusion or membrane trafficking. In Arabidopsis, the Bonsai protein is mainly associated with membranes, similar to the localization of copines in other organisms. Bon may facilitate cell growth by increasing the rate of membrane trafficking. In bon mutants the reduction in membrane dynamics could lead to smaller cells and reduced plant size.

268 Identification and analysis of T-DNA insertion mutants for two components of the chloroplast protein import apparatus

Diane Jackson Constan, Ana M. Kelly, and Kenneth Keegstra

Michigan State University-Department of Energy Plant Research Laboratory, Michigan State University

Chloroplasts must import the vast majority of their resident proteins into the organelle post-translationally. Import of these proteins is mediated by a transport complex that spans the double membrane system of the plastid envelope. Several components of this translocation machinery have been identified, including three outer envelope membrane proteins, five inner envelope membrane proteins, and one stromal molecular chaperone. Virtually all of these protein import components were originally cloned and characterized in pea. However, with the imminent completion of the Arabidopsis genome sequencing project, the Arabidopsis homologues of these components can now be easily identified as well. Genomic sequences are currently available for all of the known import components. For at least half of these proteins, multiple genomic clones can be identified, suggesting that several, if not all, of the transport components belong to multigene families.

Furthermore, ESTs representing these various genomic clones are present in the Arabidopsis database, indicating that the transport machinery may contain multiple isoforms of some components. In order to investigate the molecular functions of specific isoforms, we have obtained Arabidopsis mutants that contain T-DNA disruptions in the genes encoding selected import components, in particular Toc34 and Hsp93. These mutants were isolated from the T-DNA mutagenized Arabidopsis population available at the University of Wisconsin (<http://www.biotech.wisc.edu/Arabidopsis>). Preliminary results concerning the phenotypes of these mutants will be presented along with an analysis of the genomic sequences available for all of the chloroplast protein import components. This research has been funded by NSF and the U.S. Department of Energy.

269 Cloning and initial characterization of AtPARN, an Arabidopsis homolog of a human poly(A) ribonuclease

Mark A. Johnson¹, Jonathan T. Vogel¹, Preetmoninder Lidder^{1,2}, and Pamela J. Green^{1,3}

Michigan State University, MSU-DOE Plant Research Laboratory¹, Cellular and Molecular Biology Program², Department of Biochemistry³

Modulating mRNA degradation rates is an important means of controlling gene expression. Two structural features of mRNAs, the 5' 7-methyl guanosine cap and the 3' poly-adenosine(A) tail, are thought to protect mRNA from degradation. In fungi, animals, and plants there is evidence suggesting that removal of the poly(A) tail is an important step in mRNA degradation. The regulated removal of the poly(A) tail is the first step in the degradation of most *Saccharomyces cerevisiae* mRNAs and the rate of mRNA degradation is known to correlate with the rate of poly(A) removal. It is also known that poly(A) tail removal is the first step in the degradation of many rapidly degraded mammalian mRNAs. However, little is known about the ribonuclease(s) that are responsible for this important step in mRNA turnover. A poly(A) ribonuclease that was cloned from humans (HuPARN) is the only poly(A) ribonuclease that has characteristics consistent with a role in mRNA degradation. An Arabidopsis gene that is similar in sequence to HuPARN was identified on chromosome one and was designated AtPARN. In order to address the potential role of this gene in mRNA degradation in plants, we have cloned a cDNA corresponding to AtPARN. AtPARN and HuPARN are members of the RNaseD gene family and share three exoribonuclease domains that are thought to be necessary for catalysis. AtPARN has been expressed in *Escherichia coli* and purified by affinity chromatography. Initial experiments indicate that recombinant AtPARN is an active poly(A) ribonuclease. AtPARN mRNA is found in many plant organs, consistent with a role in a fundamental cellular process such as mRNA degradation. Future studies will make use of reverse genetic analysis in Arabidopsis to investigate the role of this enzyme in mRNA degradation in vivo. Funding for this project has been provided by the United States Department of Energy and the United States Department of Agriculture.

270 ACT7 isovariant is upregulated in response to hormones and is required for callus regeneration in Arabidopsis

Muthugapatti K. Kandasamy, Elizabeth C. McKinney, Laura U. Gilliland and Richard B. Meagher

Department of Genetics, University of Georgia

The phytohormone auxin selectively alters gene regulation and induces a wide array of changes during plant growth and development including cell division, cell expansion, cell differentiation, and organ initiation. It is widely accepted that cytoskeleton is involved in the elaboration of these responses by directing specific changes in cell morphology and cytoarchitecture. However, little has been done to demonstrate the regulation and roles of actin genes in response to auxin stimuli. In plants, an ancient and highly divergent gene family encodes actin. We have demonstrated that one of the vegetative actin isovariants, ACT7, is strongly upregulated in response to exogenous hormones in the cultured cells and organs of Arabidopsis. We also observed that the activity of ACT7-promoter, which contains several putative hormone responsive elements, was increased significantly by hormones. We tested the role of ACT7 gene in the regeneration of callus by using the act7-1 mutant allele, which showed normal plant morphology. The mutant plants were very slow in stimulating callus tissue in response to hormones and the mutant callus

contained 2-3 times lower level of ACT7 protein compared to the wild type, confirming that the ACT7 gene is essential for cell proliferation during callus formation. Moreover, there was a rapid, but transient induction of a low level of reproductive actins in the auxin induced lateral root primordia and callus tissue. Immunolabeling studies with general and subclass-specific anti-actin antibodies revealed that ACT7 and other expressed actin isoforms in the cultured cells co-polymerized to form actin microfilaments made of heteropolymers. The co-expression and co-polymerization of the predominant ACT7 with the other actin isoforms in these cells may facilitate isoform dynamics better suited for cellular responses to external stimuli such as hormones.

271 CHARACTERIZATION OF ARABIDOPSIS 68 kDa DYNAMIN-LIKE PROTEIN MUTANTS

Byung-Ho Kang, James S. Busse, Sebastian Y. Bednarek

Department of Biochemistry, University of Wisconsin, Madison, WI 53706-1569

Dynamins and dynamin-related proteins are a family of structurally related but functionally diverse high molecular weight GTP-binding proteins. Many of these proteins have been demonstrated in yeast and mammalian cells to play a role in the formation of endocytic and exocytic transport vesicles. In soybean, a 68 kDa Dynamins-like protein, known as phragmoplastin, is associated with the cell-plate. To determine the precise function of dynamins-like proteins in cell-plate formation we have identified insertional mutants in genes encoding Arabidopsis dynamins-like proteins (ADL1) related to phragmoplastin. We have isolated three recessive Arabidopsis T-DNA-tagged alleles of ADL1A. Mature homozygous *adl1A* embryos have a normal body plan but are much smaller than wild-type embryos and the mutant seeds are shrunken. In mutant *adl1A* embryos the plasma membrane and cell wall between ontogenetically related sister cells is crenulated or wavy suggesting a problem in cell-plate formation and/or plasma membrane maintenance perhaps due to a disruption in normal membrane and protein recycling. *adl1A* mutant seeds germinate and appear to undergo normal chloroplast development. However, root and shoot meristematic activity was severely limited. The *adl1A* mutants died at varying times after germination. Development of *adl1A* seedlings could be rescued in tissue culture under conditions that induce the expression of several other ADL1A-like genes. Our preliminary characterization of the *adl1A* mutants indicates that ADL1Ap is essential for normal plant development and that there is functional overlap between ADL1Ap and one or more of the other 68 kDa dynamins-like proteins in Arabidopsis.

272 The pattern of cell cycling in developing provascular strands of Arabidopsis leaves

Julie Kang and Nancy G. Dengler

University of Toronto, Department of Botany

Leaf vascular tissue functions in the import and export of water and dissolved nutrient between the leaf and the rest of the plant. These functions require both a continuous vein network (vein pattern) and mature xylem and phloem cells. This study investigated the pattern of cell cycling in: 1) provascular strand cell proliferation and in 2) differentiation of xylem and phloem cells from the provascular strands. We used a cyclin::GUS construct (*Cyc1At*, gift of John L. Celenza) to determine the pattern of cell proliferation

in developing provascular strands and the AtHB-8::GUS construct to characterize vascular cell differentiation in xylem and phloem from the provascular strands. Donnelly et al. (1999) found that the pattern of proliferative cell divisions differed between the leaf tissue layers. We observed that the duration of cell divisions in veins is correlated to vein size order. Cell divisions became restricted to the primary vein late in leaf development. We suggest that there is a correlation between the duration of cell proliferation and vein size order such that the rate of cell proliferation is extended in larger veins (primarysecondarytertiary). To investigate vascular cell differentiation, the AtHB-8::GUS construct was used as a marker for provascular cells (Baima et al., 1995). We used this construct to determine when vein pattern is formed in leaf development and to determine when provascular cells differentiate into mature xylem and phloem. A detailed characterization of leaf vein pattern development was conducted and found that vascular pattern is formed early in leaf development long before the maturation of xylem and phloem from the provascular strands. AtHB-8::GUS is present in provascular cells that have not yet differentiated, while differentiated cells do not express AtHB-8::GUS. While provascular cell proliferation and cell differentiation are separable processes, our study shows that they overlap temporally during Arabidopsis leaf development.

Baima, S., Nobili, F., Sessa, G., Lucchetti, S., Ruberti, I., and Morelli, G. (1995) *Dev.* 121: 4171-4182

Donnelly, P. M., Bonetta, D., Tsukaya, H., Dengler, R. E., and Dengler, N. G. (1999) *Dev. Biol.* 214: 407-419

273 Identification and Characterization of 5'-3' Exoribonucleases of *Arabidopsis thaliana*

James P. Kastenmayer and Pamela J. Green

Michigan State University DOE-Plant Research Laboratory and Department of Biochemistry, East Lansing, MI, 48824. kastenma@msu.edu

We are studying three 5'-3' exoribonucleases from *Arabidopsis thaliana*, AtXRN2, AtXRN3 and AtXRN4. These RNases are members of a conserved family of eukaryotic proteins; however, *Arabidopsis* has a unique number of these enzymes and is apparently lacking a member of the XRN family found in all other eukaryotes known to have XRN-like genes. These enzymes are intracellular and could be involved in cytoplasmic mRNA decay, the degradation of mRNAs during post-transcriptional gene silencing, or other RNA processing events. Their potential role in mRNA degradation is being studied to investigate a difference observed between the mRNA decay mechanisms of yeast and higher eukaryotes. In yeast, insertion of poly(G) tracts in mRNAs blocks the progression of the cytoplasmic 5'-3' exoribonuclease Xrn1p, which results in the accumulation of mRNA decay intermediates. The absence of poly(G) intermediates could be due to the ability of an AtXRN to progress directly through poly(G) tracts, or additional plant proteins, such as RNA helicases, could unwind poly(G) tracts and facilitate their degradation by an AtXRN. The AtXRNs are functional as a 5'-3' exoribonucleases that are blocked by poly(G) tracts when expressed in yeast, indicating that the second possibility is more likely. As the AtXRNs are orthologs of the nuclear Xrn2p/Rat1p of yeast, it was conceivable that they might be all targeted to the nucleus. However, through complementation of yeast mutants, and localization of AtXRN-GFP fusion proteins in

both yeast and plant cells we have determined that the AtXRNs are differentially localized in cells. Whereas AtXRN2 is targeted to the nucleus, AtXRN4 is likely cytoplasmic. AtXRN4's cytoplasmic localization is consistent with a role in mRNA degradation, and demonstrates that higher plants may be unique in that they express a Xrn2p/Rat1p ortholog in the cytoplasm.

This work was supported by grants from NSF, USDA, and DOE.

274 Visualization of tagged chromatin sites in living Arabidopsis plants

Naohiro Kato and Eric Lam

Biotech Center, Rutgers University, Foran Hall, Dudley Rd., New Brunswick, NJ 08903-0231, USA

The nucleus is the subcellular organelle in which the bulk of the genomic information within an eukaryotic cell is organized. A model of an organized subnuclear structure has emerged from studies of fixed cells. Recently, the application of the Green Fluorescent Protein (GFP) as an *in vivo* tag of genomic DNA has allowed the visualization of chromatin in live cells of animals and fungi. The chromosomes within an interphase nucleus are perceived to have a relatively static arrangement. One can expect a large rearrangement of chromosomes in the daughter cells and/or long-range movement of chromosomes in the interphase cell. It has also been shown that gene expression level can be quantitatively affected by the location of the gene in the nucleus. Thus, the arrangement of chromosomes inside the nucleus may play a role in defining the specific global gene expression pattern of each individual cell in a multicellular organism. Our objective is to contribute to our general understanding of subnuclear architecture by charting the relative physical position of genes in living plant cells.

To demonstrate the feasibility of this approach in planta, we have deployed this technology in transgenic Arabidopsis plants using the inducible gene expression system and a plant-optimized GFP-LacI fusion protein. Tandem arrays of the lac operator site are used as *in vivo* tags for insertion sites in the genome. We will present results obtained with homozygous lines of transgenic Arabidopsis. The tagged sites can be detected as bright spots of approximately 0.2 μ m diameter in the nuclei. In epidermal cells of the hypocotyl, the spot numbers are more than two times of the copy numbers of the tagged insertions in the genome, consistent with the polyploidy nature of the genome in these cells. On the other hand, in the guard cell nuclei, where the genome is consistently diploid, the spot numbers are up to or less than the copy numbers of the tags. This observation suggests that at least for the insertion sites that we have tagged, the homologous regions between sister chromatids of a diploid cell may be often paired during interphase. We will also present short- and long-term time lapse observation of the tagged sites in guard cell nuclei.

275 Arabidopsis p48, a subunit of eIF3, interacts with the CSN7 subunit of the COP9 signalosome

Tae-Houn Kim¹, Avital Yahalom², Eitan Winter², Baruch Karniol², Albrecht G. von Arnim¹ and Daniel A. Chamovitz²

¹Department of Botany, The University of Tennessee, Knoxville, TN 37996-1100, USA

²Department of Plant Sciences, Tel Aviv University, Tel Aviv, Israel

The Arabidopsis COP9 signalosome, a 550 kDa protein complex functioning in the repression of photomorphogenesis, is composed of eight subunits (CSN1 to CSN8) and is conserved among higher eukaryotes. Biochemical fractionation of the COP9 signalosome from cauliflower and protein sequencing revealed an association between the p48 protein, an ortholog of the p48 subunit of eukaryotic translation initiation factor 3 (eIF3) and the signalosome. In order to obtain a biochemical marker for Arabidopsis eIF3, we cloned the Arabidopsis ortholog of the eIF3 subunit PRT1. As expected, At-p48 and PRT1 co-fractionated by gel filtration chromatography in a protein complex of 750 kDa. In addition, however, co-immunoprecipitation experiments indicated that At-p48 is associated with the CSN7 (FUS5) subunit of the COP9 signalosome. By transient expression and immunofluorescence assays, At-PRT1 was shown to be cytoplasmic, which is consistent with its role as a bona fide component of eIF3. In contrast, At-p48 was nuclear-localized as well as cytoplasmic, similar to the localization pattern of CSN7. Interestingly, At-p48 was no longer nuclear-enriched in photosynthetically active leaves, suggesting that At-p48 localization may be correlated with activity of the COP9 signalosome. With these data, we confirmed the specific interaction between the At-p48 subunit of eIF3 and the CSN7 subunit of the COP9 signalosome. Furthermore we propose that At-p48 is a regulatory component of the eIF3 complex which might be controlled by the COP9 signalosome for proper modulation of protein synthesis.

276 Genetic analysis of quibble-1, a new trichome branch number mutation in *Arabidopsis thaliana*

Sujatha Krishnakumar, Mary Pollock, and David Oppenheimer
University of Alabama, Tuscaloosa, AL 35487

Trichomes in the leaves of *Arabidopsis* serve as a model for cell shape determination. They have a stalk and three to four branches, and are derived from a single epidermal cell. Mutational analysis has shown that the number of branches in a mature leaf trichome is under the control of positive and negative regulators. The model for the genetic control of trichome branch number proposed by Luo and Oppenheimer shows that partially redundant, parallel pathways control trichome branch number. This redundancy ensures that branch initiation can occur although there is a decrease in the branch number because of the mutation in one of the positive regulators. We have identified a new positive regulator of trichome branch number, quibble1 (qbl1). This mutation maps to the top arm of chromosome V; none of the other branch number mutations characterized thus far maps to this locus. Trichomes in qbl1-1 mutants show a decrease in trichome branch number (4% spikes, 60% two-branched, and 34% three-branched). The double mutant combinations of qbl1-1 with frc1-1, and frc3-1 indicates that they interact in an additive manner because their trichomes have an equal mix of two and three-branched trichomes. The zwi, an, and frc4-1 mutations have pronounced additive effects in combination with qbl1-1 because the trichomes in these double mutants are mostly spikes. The mutation in the negative regulator try, completely rescued the branch number defect in qbl1-1 mutation. This indicates that try does not negatively regulate qbl1-1, and that the increase in the positive regulators in try mutants can compensate for the loss of qbl1-1. Thus far, double mutant analysis with qbl1-1, supports the trichome branch model proposed by Luo and Oppenheimer, and shows that qbl1-1 is a positive regulator of trichome branch number that functions in a pathway that is independent of regulation by try. We are

currently test the endoreplication level of trichomes in *qbl1-1* to determine whether there is a reduction the endoreplication level. Trichomes in *qbl1-1* mutants are smaller in size than wildtype trichomes, but unlike *gl3*, they have a large number of three-branched trichomes. The results of this analysis, and the double mutant analysis with the other trichome mutants will be presented.

277 Control of trichome branch position by the ASYMMETRIC BRANCH loci in *Arabidopsis*

Sujatha Krishnakumar and David G. Oppenheimer

Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487-0344
USA

The differentiation of trichomes on the epidermis of the plant *Arabidopsis thaliana*, is used as a model to study how plant cells acquire specific shapes. *Arabidopsis* trichomes are large, single cells consisting of three or four branches arranged symmetrically on a stalk. We isolated seven mutations that affect trichome branch position and length without affecting branch number. These mutations identify four new genes named ASYMMETRIC BRANCH 1 (ASB1), ASYMMETRIC BRANCH 2 (ASB2), ASYMMETRIC BRANCH 3 (ASB3), and ASYMMETRIC BRANCH 4 (ASB4). In addition, some of the branches on *asb1* mutant trichomes are distorted. This phenotype is reminiscent of that seen when developing trichomes are treated with anti-actin drugs, and is similar to the distorted trichome mutants that have been shown to have an altered actin cytoskeleton (Mathur et al., 1999; Szymanski et al., 1999). Therefore, we hypothesize that the actin cytoskeleton plays a major role in trichome branch position, and that the ASB gene products regulate actin cytoskeleton function. Preliminary results suggest that the ASB genes function in the same pathways as the branch number genes. Our current working hypothesis for the control of trichome branch number is that the products of the branch number genes, AN, STA, ZWI, and the FRC genes function in a multiprotein complex that is responsible for organizing the cortical MT array in preparation for trichome branch initiation. We have also identified an unexpected gene interaction between *asb4* and *zwi* that suggests the control of trichome branch position and trichome branch number are intimately related (the ZWI gene is unique among the branch number genes in that it controls both branch number and branch position). Our results suggest that the ASB genes function with the actin cytoskeleton to position the trichome branch initiation complex.

Mathur J, Spielhofer P, Kost B, Chua N-H (1999) The actin cytoskeleton is required to elaborate and maintain spatial patterning during trichome cell morphogenesis in *Arabidopsis thaliana*. *Development* 126: 5559-5568

Szymanski DB, Marks MD, Wick SM (1999) Organized F-Actin Is Essential for Normal Trichome Morphogenesis in *Arabidopsis*. *The Plant Cell* 11: 2331-2348

278 Membrane Association of Calcium-Dependent Protein Kinases in *Arabidopsis*

Sheen X. Lu, Cynthia M. Sullivan and Estelle M. Hrabak

Dept. of Plant Biology, University of New Hampshire, Durham, NH 03824

The calcium-dependent protein kinase (CDPK) gene family in *Arabidopsis thaliana* contains more than 30 genes. Members of this group of kinases have a serine/threonine

protein kinase catalytic domain and a calcium-binding calmodulin-like domain. This structure allows these kinases to be regulated by calcium. The precise functions of CDPKs are unknown but due to the large number of isoforms, it seems reasonable to postulate that they have diverse roles within the plant. In support of this hypothesis, CDPKs have been reported in a variety of cellular locations including the cytosol, the nucleus, the cytoskeleton, and the membrane fraction. We have observed that at least 25 of the CDPK isoforms contain potential myristoylation motifs at their amino termini. Myristate is a 14-carbon fatty acid that is attached co-translationally to the amino-terminal glycine of a nascent protein. Myristoylation can facilitate membrane binding and/or protein-protein interactions. One Arabidopsis CDPK isoform, AtCPK2, has been demonstrated to be myristoylated in an in vitro transcription/translation system. Site-directed mutagenesis of the predicted AtCPK2 myristoylation site from glycine to alanine (G2A) prevents in vitro myristoylation. Using translational fusions with the β -glucuronidase (GUS) reporter gene, we demonstrated that four Arabidopsis CDPK isoforms (AtCPK1, AtCPK2, AtCPK5, and AtCPK6) are membrane associated in planta. A G2A mutation in AtCPK2 decreases, but does not eliminate, in planta membrane association. Transgenic plants containing a tagged AtCPK2 gene construct were used to determine that this isoform is associated with the endoplasmic reticulum.

279 Gene expression regulation in Arabidopsis root meristems via interstitial telomere motifs

D. Tremousaygue, A. Manevski, G. Bertoni(1), C. Bardet, B. Lescure
IFR 40, CNRS/INRA UMR 215, (1)INP/ENSAT BAP, 31326 Castanet Tolosan
FRANCE

The AAACCCTAA motif, or telo-box, corresponding to 1.3 plant telomeric repeat is over-represented within the Arabidopsis genome. These interstitial motifs are preferentially located at the 5' end of a class of genes over-expressed in cycling cells, such as genes encoding components of the translational apparatus. The role of the telo-box in gene expression regulation has been evaluated by expression analysis of a GUS reporter gene. The results demonstrate a functional synergy, in root meristems, of the telo-box with several cis-acting elements known to drive expression of genes encoding proteins involved in the translation process and also of the proliferating cellular nuclear antigen, PCNA gene. Inversely the telo-box is not required for the function of a cis-element driving the expression of the histone H4 gene. South-western screening of a lambda-ZAP library with a double-stranded telomere motif led to the isolation of a gene encoding a protein related to the animal Pur-alpha protein. Control of gene expression by telo-boxes, acting synergistically with several cis-elements, during cell cycle progression in Arabidopsis root meristems is discussed. A parallel is drawn with the function of interstitial telomeric sequences in *Saccharomyces cerevisiae*.

280 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 cell types. 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 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, FTR function is not involved in hormone-dependent cell expansion pathway. We have mapped FTR at the bottom of chromosome 1 near ADH marker and found that ftr is allelic to the botero mutant and erh3 mutant(Schneider et al., Development 124:1789-1798,1997). We have cloned the FTR gene using a map based cloning strategy. Progress in the molecular and genetic analysis of ftr will be presented.

281 Plant Nuclear Matrix/Nuclear Envelope Proteins: Plant-Specific Components of Chromatin Organization?

Iris Meier^{1,2}, Frank Gindullis², and Nancy J. Peffer²

¹Dept. of Plant Biol., Ohio State University, Columbus, OH 43220; and ²Dupont CR&D, Wilmington, DE 19880

Recently, it has been suggested that nuclear processes, such as replication, transcription, and splicing, are spatially organized and associated with a nuclear framework called the nuclear matrix, a structure of unknown molecular composition. It has been shown that chromatin is attached to the nuclear matrix via specific DNA fragments called matrix attachment regions (MARs). We have begun to dissect the plant nuclear matrix by isolating a DNA binding protein with specific affinity for MARs. We have shown that MAR binding filament-like protein 1 (MFP1) is associated with speckle-like structures at the nuclear rim that are part of isolated nuclei and the nuclear matrix. A predicted N-terminal transmembrane domain is necessary for the specific targeting of MFP1 to the speckles, indicating an association with the nuclear envelope-endoplasmic reticulum continuum. We have identified a novel protein that specifically interacts with MFP1 in yeast two-hybrid and in vitro binding assays. MFP1 associated factor 1 (MAF1) is a small, soluble, serine/threonine-rich protein that is ubiquitously expressed and has no similarity to known proteins. MAF1, like MFP1, is located at the nuclear rim and is a component of the nuclear matrix. These data indicate that MFP1 and MAF1 are components of a nuclear substructure, previously undescribed in plants, that connects the nuclear envelope and the internal nuclear matrix, and suggest a function for MFP1 in attaching chromatin to specific sites at the nuclear periphery. In animal cells the nuclear lamins, intermediate filament proteins that form a layer beneath the nuclear envelope, are involved in organizing chromatin at the nuclear rim. Since neither the fully sequenced yeast genome, nor the partially completed Arabidopsis genome contain nuclear lamin genes, it is conceivable that alternative strategies to attach chromatin to the nuclear envelope have evolved in the non-animal kingdoms, and that MFP1 and MAF1 are involved in such organization in plants.

282 Towards the cloning of PLEIADE, a gene involved in organ specific cytokinesis

Sabine Müller, Maria Heinrich and Marie-Theres Hauser
Center of Applied Genetics, University of Agricultural Sciences Vienna, Austria,
hauser@mail.boku.ac.at

Cytokinesis is a well orchestrated process leading to the separation of one cell into two daughter cells. It coordinates the partitioning of the cytoplasm which follows nuclear division. In a genetic screen for cytokinesis mutants three alleles of PLEIADE (PLE) were identified. Ple seedlings exhibit a dramatic reduction in root length and a wavy root growth pattern on nutrient agar plates, while the aerial parts are not affected. Cell biological studies revealed several alterations typical for cytokinesis mutants as multinucleate cells, cell wall stubs and synchronised cell division in incompletely separated cells. The gene was localized on the bottom of chromosome V and isolated by positional cloning. Cloning of the PLE gene was confirmed by complementation analysis of an overlapping 60 kb cosmid contig. Sequencing of all the three mutant alleles identified the mutations as single base substitutions which result in splicing defects and / or amino acid changes. The PLE gene is about 4.5 kb and consists of 12 exons leading to a transcript size of 2.6 kb. The exon / intron borders were confirmed by RT-PCR and sequencing as well as the organ specific expression. Database searches revealed that PLE belongs to a small gene family of at least eight members in *Arabidopsis thaliana*. Related genes can be found throughout the plant and animal kingdom. The significance of this common component of cytokinesis will be discussed.

This project was founded by the EU grant PL960217 FORMA.

283 VISUALIZATION OF LIVING ORGANELLES IN *Arabidopsis*

Yasuo NIWA

Lab. of Plant Cell Technology, University of Shizuoka, 52-1 Yada, Shizuoka, 422-8526,
Japan

Some organelles perform essential functions related to the dramatic changes in morphology, size, number, and subcellular location that occur during the plant life cycle. To analyze the biogenesis of organelles in higher plants, microscopic observations of living cells at various stages are necessary. Specific fluorescent dyes have made it possible to visualize some organelles; however, these dyes have problems, including photobleaching. An engineered sGFP(S65T) sequence with the favored codons of highly expressed eukaryotic proteins and with the chromophore mutation S65T has provided 120-fold brighter fluorescent signals than the original jellyfish GFP. The fusion of sGFP(S65T) with the nuclear, plastid, or mitochondrial targeting sequence, has directed the localization of sGFP(S65T) in nuclei, plastids, or mitochondria, respectively, by transient expression. In addition, sGFP(S65T) is non-toxic in *Arabidopsis*. I generated transgenic *Arabidopsis* that stably express the nuclear- plastid- or mitochondria-targeted sGFP(S65T) and could detect the fluorescent signals in each cell including root, leaf and reproductive organs. For instance, chloroplasts, etioplasts, and amyloplasts could be visualized in transgenic plants under living conditions.

284 Characterisation of an *Arabidopsis thaliana* mutant affected in cell elongation

Pagant, S. (1) , Bichet A. (1), Fagard, M. (1), McCann M. (2), Desprez T.

(1) Laboratoire de Biologie Cellulaire, INRA, Route de Saint-Cyr, 78026 Versailles cedex, France (2) Department of cell Biology, John Innes centre, Norwich Research park, Colney, Norwich, NR4 7UH, UK

How cell wall polysaccharides are synthesised and assembled in growing cells is poorly understood. We have conducted screens for EMS and T-DNA mutants with reduced hypocotyl elongation, which has yielded so far over 150 mutants. We are now focusing our analysis on mutants with reduced cell elongation and increased radial expansion. Most of these mutants have a reduced cellulose content. In this poster we present the characterisation of *kobito1* mutant which shows a dwarf phenotype in light and dark conditions.

In order to know if the dwarfism is provoked by an abnormal organisation of the microtubules, immunolabelling studies have been realised and showed that microtubules orientation is not affected. Microscopic analysis of this mutant revealed an abnormal cell wall structure. Using micro-FTIR spectroscopy, preliminary results suggest that *kobito1* is deficient in cellulose content.

Genetic analysis of a T-DNA allele revealed that the mutation is recessive, monogenic and closely linked to the T-DNA insertion which is between the markers *mi403* and *Fad7* on the chromosome 3. A plasmid-rescue strategy allowed us to obtain and sequence the genomic sequence flanking the insertion. Homology has been found in data sequences with a putative protein of Arabidopsis coming from the systematic genomic sequencing. To better understand the involvement of *KOBITO1* in cell elongation, its expression and localisation will be investigated.

285 Identification of the Arabidopsis homologue of TIMELESS gene

S. Panda, J. Hogenasch *, D. Alabadi, J.M. Alonso#, J.R. Ecker#, S.A. Kay

The Scripps Research Institute, La Jolla, CA 92037, # Plant Science Institute, Department of Biology, University of Pennsylvania, * john.hogenasch@gnf.org

In *Drosophila*, the protein TIMELESS (TIM) is a component of the central oscillator. It physically interacts with PERIOD (PER) protein and participates in the negative feedback regulation of its own transcription. It also interacts with the circadian photoreceptor CRYPTOCHROME (CRY) and mediates light induced resetting of the oscillator. Although CRY has been shown to be a circadian photoreceptor in Arabidopsis, no component of the central oscillator has been demonstrated to be conserved between plants and animals. Recently we found two Arabidopsis ESTs that bear homology to mTIM and hTIM. The ESTs (R90156, N64935) were sequenced and used to search Genbank for additional clones. The ESTs were identical, covering 2.1kb of a 3.5kb long ORF as annotated by the Arabidopsis Genome Initiative. To verify the predicted sequence and to obtain a full-length cDNA clone, an overlapping area of the N-terminal coding region was amplified by RT-PCR and the resultant fragment was sequenced. The EST and the RT-PCR products were spliced together to obtain a full-length cDNA. In parallel, we have identified a paralogue of dTIM in recently released *Drosophila* genome sequence and named it as TIMEX. Both 3'- and 5'- RACE were used to clone the full length TIMEX cDNA. The predicted aTIM protein contains four TIM Homology (TH) domains that have been previously described for mammalian and *Drosophila* TIMs. The protein contains a putative bi-partite nuclear localization signal (NLS). Significantly, aTIM contains a tetrapeptide DEDD at the extreme C-terminus. This motif is well

conserved in dTIM, mTIM and hTIM and has been implicated in cytoplasmic localization. Within these TH domains, aTIM shares significant amino acid sequence similarity with TIMELESS from *Drosophila* and mammals and with TIMEX. However, phylogenetic analyses shows aTIM, hTIM, mTIM and TIMEX to be more similar to each other than to dTIM. To find out the oscillator function of aTIM, we have screened Ecker's T-DNA insertion line collections and isolated a line containing an insertion in the aTIM gene. Currently we are testing whether aTIM plays a central clock role in *Arabidopsis*.

286 Positional cloning of TEJ; a gene affecting circadian expression of CAB2 in *Arabidopsis thaliana*

Satchin Panda, Steve A. Kay

Dept. Cell Biology, The Scripps Research Institute, 10550, North Torrey Pines Road, La Jolla, CA 92037

tej, a recessive mutation that maps to chromosome 2, affects the circadian control of CAB gene expression. tej lines exhibit a waveform of cycling bioluminescence with higher amplitude and a free running period that is 2hrs longer than the wild-type parent (tej in Sanskrit means bright). Endogenous CAB mRNA levels oscillate with a long period in tej, confirming the period lengthening effect of the mutation is not specific to the reporter transgene. The mutant also alters photoperiod-sensitive initiation of flowering. The circadian phenotypes of tej are evident in different fluence levels of either red or blue light suggesting no general defect in photoperception. Given these findings and the potential importance of TEJ in the circadian function of *Arabidopsis* we took a positional cloning approach to isolate the TEJ gene. The TEJ locus was delimited to an interval of 110 Kb between nga361 and COP1 on Chromosome 2. Rescue of the mutant phenotype with cosmid clones and sequencing of predicted ORFs in this interval led to identification of TEJ gene. The mutation causes an amino acid change in the predicted protein. The predicted protein shows extensive similarity to poly-ADP ribosyl glycohydrolase (PARG) from mammals, *Drosophila* and *C. elegans*. Poly (ADP-ribosyl)ation represents one of the posttranslational modifications of nuclear proteins and is achieved by the enzyme poly(ADP-ribosyl) polymerase (PARP). PARP uses NAD⁺ to synthesize both linear and branched homopolymer of ADP-ribose residues. PARG hydrolyzes the polymer to produce free ADP-ribose residues. Poly(ADP-ribose) metabolism in mammals has been associated with chromatin decondensation, DNA repair, programmed cell death and regulation of DNA binding activity of transcription factors. The putative enzymatic nature of TEJ protein suggests that poly(ADP-ribose) metabolism may be involved in *Arabidopsis* clock function.

287 Characterization of mangled, a novel *Arabidopsis* embryo mutant

Rojo E.", Gillmor S. *, Somerville C.R. * and Raikhel N.V."

"MSU-DOE PRL and *Carnegie Institution of Washington

We have isolated a novel *Arabidopsis* embryo mutant, mangled, which arrests at a size smaller than a heart stage wild type embryo. Interestingly, the ovule size is also reduced to less than half of wild type. Moreover, mangled embryos have multinucleated cells and underdeveloped cell walls as well as an aberrant cell division pattern, suggesting that cell wall formation may be affected. We have isolated the MANGLED gene, which is localized in the lower arm of chromosome II, from a T-DNA tagged line. The T-DNA is

inserted in the fourth exon of the gene. The MANGLED gene encodes a predicted 97 kDa protein, similar to the yeast VPS16 gene. In *S.cerevisiae* the VPS16 gene is required for vacuolar protein sorting as well as for vacuole biogenesis. We will present data on the expression, localization and possible role of the MANGLED protein in plant development.

288 NMP1 - a Potential Component of the Internal Plant Nuclear Matrix

Annkatrin Rose^{1,2}, Frank Gindullis², and Iris Meier^{1,2}

¹ Dept. of Plant Biol., Ohio State University, Columbus, OH 43210; and ² DuPont CR&D, Wilmington, DE 19880

The nuclei of eukaryotic cells contain an insoluble filamentous meshwork, called the nuclear matrix, which is believed to be involved in organizing chromatin, as well as in different aspects of nucleic acid metabolism. Specific DNA fragments that attach chromatin to the nuclear matrix have been identified and called matrix attachment regions (MARs). While the plant nuclear matrix has similar characteristics to the animal one, none of the known animal nuclear matrix proteins have been found in plants. MAR binding filament-like protein 1 (MFP1) was the first plant nuclear matrix protein cloned. Here, we describe the cloning of a novel filament-like plant protein (nuclear matrix protein 1, NMP1) from tomato in a yeast two hybrid screen performed with the central domain (aa 83 to 491) of MFP1. The NMP1 cDNA encodes a putative protein of ca. 37 kD which is highly α -helical with multiple stretches of short amphipathic, predicted coiled-coil regions and has a charge profile with alternating basic and acidic regions. Data base searches indicated that NMP1 is a novel protein with no homologs in animals and fungi, but is highly conserved among flowering and non-flowering plants, indicating a plant-specific function of NMP1. NMP1 mRNA and protein could be detected in all tissues analysed. Western blot analysis, immunolocalization and in vivo localization of a GFP-NMP1 fusion protein showed that NMP1 is localized both in the cytoplasm and the nucleus of the plant cell and that the nuclear fraction of NMP1 is associated with the insoluble nuclear matrix. No DNA-binding activity could be detected for NMP1, but interestingly NMP1 was found to be capable of activating reporter gene expression in yeast when targeting an GAL4 DNA binding domain-NMP1 fusion protein to a minimal promoter containing GAL4 binding sites. Transient co-transformation of tobacco NT-1 cells with the GAL4 DNA binding domain-NMP1 fusion construct and a GUS reporter gene under the control of a plant minimal promoter containing GAL4 binding sites increased the GUS activity approximately two-fold. This result indicates that NMP1 can also act as a transcriptional activator when targeted to a plant minimal promoter.

289 Organized cell division and expansion during embryogenesis requires FACKEL, a sterol C-14 reductase

Kathrin Schrick¹, Ulrike Mayer¹, Andrea Horrichs², Christine Kuhnt³, Catherine Bellini⁴, Jeff Dangl², Jürgen Schmidt³, and Gerd Jürgens¹

¹ZMBP, U. of Tübingen, Tübingen, Germany, ²Max Delbrück Lab., Köln, Germany (present: Dept. of Biology, U. of North Carolina, Chapel Hill), ³Leibniz-Institut für Pflanzenbiochemie, Halle, Germany, ⁴INRA, France

The Arabidopsis embryo consists of growing populations of cells whose fates are determined in a position-dependent manner to form the adult organism. Mutations in the

FACKEL (FK) gene affect body organization of the seedling. Here we report that FK is required for cell division and expansion and is involved in proper organization of the embryo. We isolated FK by positional cloning. Expression analysis in embryos revealed that FK mRNA becomes asymmetrically distributed at the heart stage and in mature stages is localized to meristematic zones. FK encodes a predicted integral membrane protein related to sterol reductases across species, including the vertebrate lamin B receptor and yeast sterol C-14 reductase ERG24. We provide functional evidence that FK encodes a sterol C-14 reductase by complementation of the yeast *erg24* mutant. GC/MS analysis of sterols confirmed that *fk* mutations lead to accumulation of intermediates in the biosynthetic pathway preceding the C-14 reductase step. Although *fk* represents a sterol biosynthetic mutant, the phenotype was not rescued by feeding with brassinosteroids (BRs), the only plant sterol signaling molecules identified so far. We present a model in which the synthesis of sterol signals in addition to BRs is important for regulated cell growth and organization during embryonic development. Our results indicate a novel role for sterols in the embryogenesis of plants.

290 Activation tagging of genes involved in trichome branching in *Arabidopsis*
Jeanne M. Serb, Mary A. Pollock, and David G. Oppenheimer
University of Alabama

Arabidopsis trichome development is being used in our lab to study the genetic and molecular basis of plant cell shape. Genetic screens for plants with altered trichome shape have resulted in the identification of over 35 genes involved in trichome morphogenesis. One of these genes, ZWICHEL (ZWI), encodes a novel, kinesin-like calmodulin-binding protein. Mutations in ZWI produce stalkless, two-branched trichomes. We are using activation tagging to discover new and interesting genes involved in processes of trichome branch initiation. We are transforming *zwi* mutant plants with the activation tagging vector, pSK115, to generate a tagged population. The population will be screened for plants showing rescue of the branch number defect of *zwi* mutants. Any new genes discovered by this approach will provide new information on the function of ZWI and of the branching process within trichomes. The mechanisms controlling trichome shape can eventually be used as a paradigm for localized cell expansion.

291 Clonal analysis of stomatal development and patterning in *Arabidopsis* leaves
Serna, L., Torres, J. and Fenoll, C.
Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-la Mancha, Toledo, Spain

Cell lineage has been used to explain the stomatal distribution in several plant species. We have used transgenic plants carrying a 35SGUS::Ac construct that produces clonal sectors to test the possible role of cell lineage during the establishment of stomatal patterning in *Arabidopsis* leaves. The analysis of sectors ranging from two to five cells indicates that most of the stomatal complexes derive from a single and immediate precursor cell through a stereotyped pattern of cell divisions. These sectors also reveal the direction of the cell division sequence: both clockwise and anticlockwise patterns take place during stomatal development. Moreover, considering that stomatal complexes exhibit a clustered patterning in *Arabidopsis* leaves, its monoclonal origin allows to the cell lineage-derived mechanism to explain the cell distance between stomata.

292 Expression of a fungal cutinase in transgenic Arabidopsis plants leads to postgenital fusions of organs

Patrick Siebera, Martine Schordereta, Ulrich Rysera, Antony Buchalaa, Pappachan Kolattukudyb, Jean-Pierre Métrauxa, and Christiane Nawratha

a Department of Biology, University of Fribourg, CH-1700 Fribourg, Switzerland, b

Departments of Biochemistry and Medical Biochemistry and Neurobiotechnology Center, The Ohio State University, Columbus 43210, Ohio, USA

A major structural component of the cuticle of plants is cutin. The functional analysis of cutin in vivo has been limited because no mutants with specific defects in cutin have been characterized. Therefore, transgenic Arabidopsis plants that express and secrete a cutinase from *Fusarium solani* f sp pisi were generated. Arabidopsis expressing the cutinase in the extracellular space show an altered ultrastructure of the cuticle and an enhanced permeability of the cuticle to solutes. These differences coincide with strong postgenital organ fusions. The junctions of the fusions contain pectic polysaccharides. As fused organs grow apart from each other, organ deformations and protrusions composed of epidermal cells develop at positions with high mechanical stress. These results demonstrate that an intact cutin layer is not only important for plant-environment interactions, but also prevents fusions between different plant organs and is, therefore, necessary for normal epidermal differentiation and organ formation.

293 Arabidopsis seed coat development: morphological differentiation of the Arabidopsis outer integument

V. Vaughan Symonds, J. Brian Windsor, John Mendenhall, Alan M. Lloyd

Molecular Cell and Developmental Biology Section, The University of Texas at Austin, Austin, Texas 78712-1095

A morphological description of the differentiation of the outer integument of the Arabidopsis thaliana seed is presented. The period covered starts at about the octant embryo stage, extends to the mature seed, and concludes beyond that at the initial stages of seed imbibition. During this period the two-celled-layered outer integument goes through a dramatic differentiation process. The outer cell layer secretes mucilage in a ring between the plasma membrane and the outer cell wall at the corners of the cell. This secretion forces the cytoplasm into a columnar shape in the center of the cell. Before and during this process, starch granules are produced, initially at the center of the outer wall and later within the column. Late in differentiation, the starch granules are degraded as the cell produces a highly reinforced wall surrounding the columnar protoplast and at the radial walls between adjacent cells. This results in a cell containing large amounts of mucilage surrounding and completely outside of a highly reinforced columella. The mucilage and outer wall then dehydrate to leave the columella and radial walls visible as the epidermal plateau and reticulations visible on the mature seed. The inner cell layer of the outer integument also produces and degrades starch granules concomitantly with the outer layer but produces no mucilage. In the mature dry seed the collapsed outer wall remains connected to the top of the columella and the radial walls but these connections are rapidly broken as the mucilage fully hydrates. Arabidopsis thaliana

294 Cell shape mutants offer insight into cytoskeletal function

Dan Szymanski
Purdue University

Great progress has been made in identifying genes that transduce information from endogenous or environmental cues into coordinated plant developmental responses. In many cases the outcome of the signaling event is a change in cell structure, and presumably in cytoskeletal organization. However, very little is known about the cellular intermediaries that link signal input to cytoplasmic reorganization. To increase our understanding of how cytoskeletal organization is regulated, we are conducting a genetic analysis of epidermal cell morphogenesis. Trichome development has been used as a model process to study the control of cell fate and pattern formation in the leaf. Recent molecular genetic analyses of trichome initiation suggest the activity of a multimeric complex of transcriptional regulators is a key point of regulation. A cell biological examination of trichome morphogenesis has been initiated to understand how transcription factor activity leads to changes in cell shape. Because trichome growth includes many diverse and conserved growth processes, it is also an ideal genetic system to study relevant aspects of cell and tissue organization. Pharmacological and immunolocalization data suggest that a clear hierarchy of cytoskeletal function operates during the early stages of trichome formation: microtubule-dependent activity is required to initiate cell polarization events, while actin microfilaments are required to maintain and coordinate an established growth pattern. Pharmacological agents that disrupt microfilaments phenocopy the "distorted" class of trichome mutants. An analysis of cell shape and microfilament structure in wild type, mutant, and drug-treated trichomes is consistent with a role for actin in the maintenance and coordination of an established growth pattern. Genetic screens for pleiotropic "distorted"-like trichome mutants identified a seedling lethal mutation in the SPIKE gene that causes widespread defects in epidermal cell and tissue organization. SPIKE shares amino acid sequence identity with a family of proteins present in humans, flies, and worms that control plasma membrane-localized microfilament organization and tissue morphogenesis. Analysis of the SPIKE gene may provide insight into cytoskeletal reorganization, cellular morphogenesis, and tissue development in plants.

295 Functional analysis of the receptor-like kinase gene expressed in both shoot and root apical meristems

M. Takemura, E. Tani, Y. Takeda, A. Yokota and T. Kohchi

Nara Institute of Science and Technology

We have isolated a receptor-like kinase gene, IMK3 from an equalized cDNA library of Arabidopsis inflorescence meristem by differential screening. So far, the predicted IMK3 protein was found to contain an extracellular receptor domain consisted of a leucine-rich repeat, a transmembrane domain and a cytoplasmic serine/threonine kinase domain.

Previous works showed that IMK3 promoter::GUS transgene was expressed not only in the shoot apices but also in root tips during both vegetative and reproductive phases. In this study, we analyzed expression patterns of the GUS driven by the IMK3 promoter in details. Results showed that GUS expressions have been also detected in embryos. To confirm the expression patterns of IMK3, we have further performed RT-PCR analysis with various tissues at various developmental phases. Results indicated that IMK3 mRNA was detected in the tissues which contained either shoot apices or root tips

throughout development. These results suggested that IMK3 was expressed in the meristematic cells of both shoots and roots and involved in their functions during plant development.

To ascertain whether IMK3 is indeed a protein kinase, the kinase domain of IMK3 (IMK3KD) fused with GST was expressed in *E. coli* and examined its autophosphorylation activity. The GST-IMK3KD protein showed a single phosphorylated band which corresponded to the GST-IMK3KD, only when Mn²⁺ was present.

To elucidate the signal transduction pathways which IMK3 function, we have isolated three candidates which interact with the kinase domain of IMK3 by the yeast two-hybrid screening. These candidates were AGL24 which is a member of MADS-box genes, RPB6L which shows a homology to the RNA polymerase subunit 6 gene, and UNK which has no similarities with any known gene. Next, we examined the significance of their interactions by *in vitro* binding assays. As a result, AGL24 bound strongly with IMK3 kinase domain, whereas UNK bound weakly. RPB6L did not bind at all. In addition, AGL24 was phosphorylated by IMK3KD, indicating that AGL24 is the most likely to be the target for IMK3 kinase domain. We are also investigating the expression pattern and subcellular localization of candidates isolated. Here we will report the molecular analysis of these three genes.

296 Characterization of Arabidopsis mutants with left-handed spiral

Thitamadee Siripong, Tuchihara Kazuko, Hashimoto Takashi

Graduate School of Biological Science, Nara Institute of Science and Technology (NAIST), JAPAN

A recessive mutant of *Arabidopsis thaliana*, *spiral1(spr1)* shows right-handed spiralling in the epidermal cell files of root, etiolated hypocotyl and dark-grown stem. We mutagenized *spr1* with EMS, and screened the M2 population for mutants in which the original right-handed spiral was reversed to the left-handed spiral. *lefty1(lft1)* and *lefty2(lft2)* are semi-dominant in the *spr1* background, but *lft1* is recessive in the *Spr1* background. *lft1* and *lft2* are hypersensitive to taxol and propyzamide, and are mapped on chromosomes 4 and 1, respectively. Cortical microtubule arrays in *lft1* root epidermal cells at the elongation zone are arranged in right-handed helix. A mis-sense mutation was identified in a candidate LFT1 gene. This putative *lft1* mutation supports our microtubule-dependent model on the spiral phenotype.

297 A Gene Family Containing Two Putatively Functional Members Encodes the L3 Ribosomal Protein in Arabidopsis

Michael Tilley and Randy Scholl

Ohio State University

Ribosomes are essential cellular components composed of ribosomal RNA (rRNA) and proteins (rProteins) in eukaryotic ribosomes there are 80 or more rProteins per ribosome. Ribosomes have been studied in some detail in bacteria, yeast and other organisms, and to a lesser degree in plants and it is known that their main cellular function is to translate mRNA and synthesize proteins. It has also been shown that stoichiometric amounts of each rProtein must be present in order for ribosomes to assemble efficiently. Therefore, rProtein genes have been the subjects of studies in coordinated gene regulation.

In *E. coli* each rProtein is encoded by a single gene, in *Saccharomyces* each protein is encoded by a single gene or a two-member gene family. In plants there is strong evidence that each protein is encoded by a small gene family with two or three members. However, the deduced amino acid sequence of each member is very similar, and in some instances differs by only a single amino acid. This has made it difficult to determine if products from all of the gene families are incorporated into ribosomes and if some of the family members have specialized roles.

We have characterized two ribosomal protein genes from *Arabidopsis* that encode a homolog of the *E. coli* L3 protein. These two genes are relatively divergent, sharing approximately 75% nucleotide identity and 85% amino acid identity. These genes have been named Arp1 and Arp2. Arp1 appears to be constitutively transcribed at high levels in all tissues. Its mRNA is polyadenylated and can be isolated from the polyribosome fraction. Arp2 is also transcribed in all tissues analyzed thus far, however, its mRNA is found in the poly A- fraction after separating poly A+ and poly A- RNA on an oligo-dT column. Sequence analysis and RT-PCR using an oligo-dT primer for first strand synthesis has located a putative polyadenylation site and at this site a short polyA tail may be present on a fraction of the messages. Arp2 mRNA can also be isolated from polyribosomes, indicating that at least a fraction of it is translated.

298 Targeted misexpression for investigating cellular interactions during *Arabidopsis* development

Elisabeth Truernit¹, Kirby Siemering², Sarah Hodge¹ and Jim Haseloff¹

¹Department of Plant Science, University of Cambridge, Downing Street, Cambridge, UK; ²ForBio Research, 50 Meiers Road, Queensland, Australia 4068

Cell fate in plants shows a high degree of plasticity and self-organisation. There is growing evidence in plants that cell fate is determined by position rather than lineage (Steeves and Sussex, *Patterns in Plant Development*, 1989; Scheres, *Curr. Opin. Gen. Dev.* 7, 501 - 506, 1997). Positional information is most likely to be obtained by cell to cell communication, giving rise to a network of local cellular interactions in the plant body.

In order to examine cell fate and cell interaction in *Arabidopsis thaliana*, our lab has developed a system to precisely alter and monitor the behaviour of particular cells in a non-invasive way in the living plant. To this end a library of transgenic *Arabidopsis* lines has been created by *Agrobacterium* mediated transformation. Each of these plant lines contains a randomly inserted gene for a foreign transcription factor (mGAL4-VP16), and adjacent genomic DNA sequences confer specific patterns of mGAL4-VP16 expression in these plants. Since the inserted T-DNA also contains a GAL4 responsive gene encoding the green fluorescent protein (GFP), expression of GAL4 can be easily visualized by fluorescence. With these plant lines in hand, any gene of interest can be expressed in a distinct, GAL4 mediated way. We simply have to cross a transgenic plant containing this particular gene under the control of GAL4 upstream activation sequences (UAS) with a plant line that expresses GAL4 in the desired cells.

We are using this system to better map cell cell interactions during *Arabidopsis* development. We have crossed plants harbouring a UAS-construct that encodes the A-chain of diphtheria toxin (DTA) into our GAL4-lines. DTA is a potent toxin that kills plant cells when expressed. Thus we can ablate cells in a cell or cell type specific way in

our system. This allows us to probe directly the process of reorganisation of the plant body. We also have started to misexpress Arabidopsis homeodomain proteins in our system. We are studying their potential for altering cell fate and effects on neighbouring cells.

299 SLATs: A family of Arabidopsis proteins resembling components of the RAS signaling pathway

Daniel M. Vernon, Martha D. Brinck, Marianne A. Brady, Jennifer H. Eastberg, & Nancy Forsthoefel

- Biology Department, Whitman College, Walla Walla, WA 99362

Leucine-Rich Repeat proteins (LRR-Ps) are a superfamily of eukaryotic proteins that contain tandem repeats of a 23-25 amino acid leucine-rich motif. Many LRR-Ps have roles in cell signal transduction. A number of LRR-Ps from plants have been characterized; most are pathogen resistance proteins, or receptor kinases with extracellular LRR domains. We have characterized 8 Arabidopsis genes encoding a novel class of plant LRR protein. Members of this class are distinguished by their leucine-rich consensus motif, which differs in several respects from those of other plant LRR-Ps. Based on this motif structure, these proteins more closely resemble animal LRR-Ps involved in RAS signaling, including *C. elegans* SUR8 and mammalian RSP1. We have therefore named these polypeptides SLATs, for SUR8-like LRRs of Arabidopsis thaliana. All SLAT proteins contain an internal domain consisting of 8.5-10.5 tandem repeats of the leucine-rich motif, bracketed by variable N- and C-terminal domains. These variable domains share some common features, including low-complexity N-terminal stretches and clusters of charged residues in the C-terminus. Sizes range from 380 to 549 amino acids; differences are largely due to differences in the length of the N-terminal domains. SLATs fall into 3 subclasses based on protein and gene structure. All are predicted to be intracellular proteins. SLAT mRNAs are widely expressed during development, although some are present at very low levels. Prospective SLAT orthologues from several crop species are represented in gene, protein, and EST databases, indicating that these proteins are widespread in plants. SLATs represent an intriguing new class of plant polypeptides that may serve as components of intracellular signaling pathways. The functions of these proteins in plant growth and development will be investigated by molecular analysis and reverse genetics. -Supported by the NSF and Whitman College

300 What is the biological significance of the cytokinesis-specific expression of the KNOLLE syntaxin?

Axel Völker, Stefan Mangold, Niko Geldner, Wolfgang Lukowitz* and Gerd Jürgens
ZMBP, Entwicklungsgenetik, Universität Tübingen, Auf der Morgenstelle 1, 72076 Tübingen, Germany; * Present address: Dept. of Plant Biology, Carnegie Institution, Stanford, CA, USA

During plant cytokinesis, Golgi-derived vesicles traffic to the plane of division where they fuse with one another to form the cell plate. Fusion of cytokinetic vesicles is impaired in Arabidopsis knolle mutants. The KNOLLE (KN) gene is expressed in a cell cycle-regulated manner and encodes a cytokinesis-specific syntaxin which accumulates during M phase and is localised to the plane of division (Lukowitz et al., 1996, Cell 84, 61-71; Lauber et al., 1997, J. Cell Biol. 139, 1485-1493). To analyse the significance of

this tight regulation, we misexpressed the KN cDNA under the control of the AP3 and CaMV 35S promoters. AP3::KN transgenic plants were phenotypically normal although KN protein was detected in petal extracts as expected (Jack et al., 1994, Cell 76, 703-716). 35S::KN transgenic plants also did not show any obvious phenotypic abnormality. KN protein was detected in several plant organs, including leaves which normally do not accumulate KN protein (Lauber et al., 1997). To determine why 35S::KN appeared to have no biological effect, we analysed the ability of this construct to rescue knolle mutant embryos. In contrast to genomic fragments spanning the KN gene and adjacent intergenic regions, the 35S::KN construct was unable to rescue. In situ hybridisation to normal and knolle mutant embryos indicated that the 35S promoter was weakly active at later stages of embryogenesis as compared to the strong expression conferred by the KN promoter. In postembryonic development, 35S::KN expression yielded high levels of membrane-integrated KN protein which, in the root, preferentially accumulated in non-dividing cells outside the root meristem. Our results suggest that ectopic expression of KN protein does not interfere with non-cytokinetic vesicle trafficking pathways. In contrast to the endogenous KN promoter, the 35S promoter seems to be not strong enough during embryogenesis to yield the high levels of KN protein required at a specific time during the cell cycle for cytokinesis to occur.

301 A T-DNA INSERTION MUTANT IN ARABIDOPSIS WITH A SMALLER SHOOT APICAL MERISTEM

Chongying Wang^{1,2}, Malin Persson¹, S. Mahdi Seyedi¹ and Olof Olsson¹

¹Department of Cell and Molecular Biology, Goteborg University, P.O. Box 462, SE 405 30 Goteborg, Sweden

²Department of Biology, School of Life Science, Lanzhou University, 730000 Lanzhou, P.R.China

In higher plants, above ground growth and development is regulated by the shoot apical meristem (SAM) since leaves, stems, and flowers are generated from this meristem. In the SAM itself a delicate balance between cell proliferation and cell differentiation is required to maintain a functional SAM. We recently identified an Arabidopsis mutant by T-DNA insertion mutagenesis, in which the SAM was smaller and the transition from vegetative phase to reproductive phase was about one week later than in wild type.

Typical phenotypic changes of the mutant were smaller siliques and darker green rosette leaves with shorter petioles, and rounder, smaller leaf blades. In addition, the number of stamens and internodes was decreased. Anatomical analysis of the mutant confirmed that it had a smaller and flatter SAM, which showed less mitotic activity, especially in young seedlings.

A plant promoter activated a uidA reporter gene in the T-DNA. GUS analysis in whole plants revealed that this promoter was active at SAM and in vascular tissues of leaves, stems and roots. GUS staining of mutant inflorescence stem sections demonstrated that only procambium, and new xylem cells derived from the procambium, displayed GUS activity. Sequence analysis of DNA flanking the T-DNA insert revealed that the T-DNA had been inserted in chromosome 4 at a site corresponding to 29 amino acids upstream the C-terminal part of a putative protein. So far, no similarities between this protein and any other protein in the data bases has been found.

Taken together, our results suggest that the gene tagged by T-DNA could encode a novel protein, which may be involved in the intricate regulation of the size of the proliferating cell population in the SAM. Work is now in progress to more exactly define the molecular activity of this gene.

302 Microtubule organization and cell shape regulation: a shifting paradigm

Geoffrey Wasteneys¹, Keiko Sugimoto¹, Madeleine Rashbrooke¹, Tobias

Baskin², Richard Williamson¹, Takashi Hashimoto³, Herman Hoefte⁴

¹Plant Cell Biology, RSBS, Australian National University, Canberra, Australia;

²University of Missouri, Columbia, USA; ³Nara Institute of Science and Technology,

Nara, Japan; ⁴INRA, Versailles, France

Cortical microtubules are major control agents in plant morphogenesis and molecular-genetics is helping to unravel their exact function. Mutations at the MOR1 locus cause temperature-sensitive cortical microtubule disorganization, leading to reduced anisotropy, organ spiralling, root hair deformation and abolition of fertility. MOR1 functions in a hormone-independent manner and does not appear to control microtubule arrays involved in the cell cycle. Radial swelling occurs despite cellulose microfibrils remaining transverse, an effect partially phenocopied by drugs like oryzalin and taxol that affect microtubule dynamics. These results, along with genetic analysis of interactions between the cellulose-deficient *rsw1* mutant and *mor1*, question the way microtubules are thought to regulate growth anisotropy.

We are now investigating how MOR1 interacts with other microtubule organization and morphogenesis genes. The *botero1* mutants have constitutive random cortical microtubule organization in root epidermal cells, and decreased growth anisotropy. The phenotype of *bot1mor1* double mutants is additive, suggesting the two genes regulate independent aspects of microtubule organization. Mutant alleles of the two SPIRAL loci confer right handed spiralling of roots, hypocotyls and petioles. Opposite, left-handed spiralling occurs in *mor1* mutants at 31°C. The *mor1-1spr1-1* double mutants resemble *spr1* homozygotes at 21°C but at 31°C the direction of spiralling is quickly reversed and the *mor1* phenotype dominates. In contrast, MOR1 appears to be fully epistatic to SPR2; genotypic double mutants are indistinguishable from *mor1* homozygotes with normal growth at 21°C and the typical *mor1* phenotype at 31°C.

303 Arabidopsis RopGAPs Act as Negative Regulators in Rop GTPase Signaling and

Require the Cdc42/Rac-Interactive Binding Motif for Rop-Specific GTPase Activation

Guang Wu, Hai Li and Zhenbiao Yang

Ohio State University and California University at Riverside

We describes a unique function for the Cdc42/Rac-interactive binding (CRIB) motif in the regulation of novel GTPase-activating proteins, termed RopGAPs. RopGAPs activate the GTPase activity of Rop, Cdc42/Rac-related GTPases, which plays an important role in the regulation of tip growth and many other processes in plants. Besides GAP domain, RopGAPs contain a CRIB motif known to allow Cdc42/Rac effectors to bind active Cdc42/Rac. RopGAP overexpression in Arabidopsis pollen inhibits pollen tube growth, as *Rop1* antisense RNA and dominant negative *rop1* mutants do, indicating that RopGAP acts as a negative regulator. The function of the CRIB motif in RopGAPs was then studied using CRIB point mutations and domain deletion mutants. Compared to wild type

RopGAPs, both mutants have drastically reduced capacities to stimulate Rop GTPase activity and to bind Rop. Wild type RopGAP1 has a weak GAP activity on Cdc42, similar to that of the RopGAP mutants on Rop. These results suggest a CRIB-dependent mechanism for determining the specificity of RopGAPs for Rop. RopGAP CRIB interacts with both GTP- and GDP-bound Rop and its transitional state, whereas the GAP domain specifically interacts with GTP-Rop as wild type RopGAP does. This study reveals a novel GAP-regulatory mechanism by which CRIB specifically promotes GAP-mediated Rop GTPase stimulation.

304 Understanding spindle function and assembly by analyzing microtubule structures in the ask1-1 mutant

Ming Yang & Hong Ma

Dept. of Biology and LSC, Penn State University, University Park, PA 16802

The Arabidopsis ASK1 protein affects diverse cellular processes including homologue separation in male meiosis, possibly by controlling the concentrations of other regulatory proteins via ubiquitin-mediated proteolysis. In ask1-1 mutant male meiosis, homologues exhibit nondisjunction during anaphase I, resulting in uneven distribution of chromosomes in the microspores. Because the spindle and chromosomes are intimately associated during cell division, the nondisjunction of homologues in ask1-1 may influence spindle function and assembly. Thus, we examined the microtubule structures during and after male meiosis in wild-type and ask1-1 plants. At metaphase I, the mutant spindle was morphologically indistinguishable from that of the wild type. But at anaphase I, it required much more spindle elongation in the mutant than in the wild type, to just stretch the homologues and/or pull them away from the equator. These data suggest that stronger force is needed for moving the homologues. During meiosis II in the mutant, spindle-like structures co-localized with scattered chromosomes. In extreme cases, when only one group of chromosomes was seen in the cell, one spindle was formed and co-localized with these chromosomes. These results demonstrate in vivo that chromosomes play a role in spindle assembly. Other unusual microtubule structures found in mutant interphase microspores include, in the order of frequency, microtubule aggregates in constricted regions (12%), partial-spindle-like structures (PSLS, 4%), curved microtubule bundles including rings of microtubules (4%), and globular structures (GS, 2%). The PSLS had a fixed length of 6.3 micrometers. The curved or ring structures had variable lengths but are often longer than the PSLS. When the cells were subject to incubation in buffer for half an hour before fixation, the number of GS increased while the numbers of other structures decreased, suggesting that other structures might be disassembled to form GS. Since the features of these structures resemble those found in single microtubules assembled and disassembled in vitro, they might represent various steps of spindle assembly and disassembly in vivo. Thus, the ask1-1 mutant may provide a useful tool for understanding normal in vivo spindle assembly.

305 Antisense Expression of the Stromal Processing Peptidase Gene in Arabidopsis Disrupts Chloroplast Biogenesis and Plant Development

Rong Zhong, Rongguan Jin, Stefan Richter, and Gayle Lamppa

Department of Molecular Genetics and Cell Biology, The University of Chicago, 920 E. 58th St., Chicago, IL 60637, USA

We have characterized a chloroplast processing enzyme that cleaves an array of precursors, and thus functions as a general stromal processing peptidase (SPP). SPP is highly conserved, showing ~80% sequence identity between pea and Arabidopsis. Downregulation of SPP *in vivo* was investigated to determine how chloroplast biogenesis and plant development are affected. An antisense gene for SPP was expressed in tobacco and Arabidopsis. Expression of the antisense gene in Arabidopsis was especially severe, probably because Arabidopsis has one SPP gene, whereas tobacco has multiple copies. In Arabidopsis, growth was retarded, and leaf morphology was irregular (curly or chewed), with intermixed green and albino sectors. The tobacco phenotype was different; leaves were normally shaped and uniformly chlorotic. Chloroplast numbers were low in Arabidopsis, there were fewer grana stacks, and unusual amounts of starch or lipid accumulated in the chloroplast. Downregulation of SPP in tobacco inhibits import of proteins in chloroplasts isolated from the transgenic plants. We have now investigated the fate of a ferredoxin transit peptide-GFP fusion protein (fTP-GFP) in the same transgenic plants *in vivo*. Using protoplasts from wild type tobacco, we found that GFP is imported by the ferredoxin transit peptide. However, in plants expressing the antisense gene, GFP accumulates on the exterior of chloroplasts. Fluorescent clusters are observed in a punctuated pattern. Not all chloroplasts show the same pattern, suggesting that there are stages in chloroplast biogenesis when SPP levels are most critical. fTP-GFP was introduced into the Arabidopsis transgenic plants, and studies are underway to examine the location of GFP in these mutants. The pleiotropic effects of downregulating SPP expression emphasize the critical role of SPP in chloroplast biogenesis and plant development. SPP does not function in isolation as precursors are imported, but its activity influences efficient protein transport into the organelle.

306 The Floral Inducer FT

Ji Hoon Ahn and Detlef Weigel

Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037; ahn@salk.edu; <http://www.salk.edu/LABS/pbio-w/>

Using activation tagging (Weigel et al., *Plant Physiol.* 122:1003 [2000]), we isolated the floral inducer FT (Kardailsky et al., *Science* 286:1962 [1999]). *ft* loss-of-function mutants flower late, while FT overexpression causes early flowering regardless of photoperiod, indicating that FT levels are critical in determining flowering time. We also found that FT is more highly expressed in long days than in short days, and that the increased expression in long days is dependent on CONSTANS (CO). These observations are consistent with a role of FT downstream in the photoperiod-dependent pathway of floral induction. Surprisingly, FT expression was also reduced in young *fca-2* seedlings, suggesting that FT acts also partially downstream of the autonomous pathway of floral induction. However, FT expression was unchanged in gibberellin-deficient *ga1-3* seedlings, which is in contrast to the floral meristem-identity gene LEAFY (LFY), which acts in parallel with FT and which is a target of gibberellin-mediated floral induction (Blázquez and Weigel, *Nature* 404:889-892 [2000]).

FT is a member of small gene family that includes at least six genes in Arabidopsis. The only other gene with a known role is TERMINAL FLOWER 1 (TFL1), a potent inhibitor of flowering (Bradley et al., *Science* 275:80 [1997]). We have isolated insertion alleles in two other members, ATC and MFT, but neither mutant shows an apparent phenotype.

This work has been supported by fellowships from KOSEF and the Hoffmann Foundation (J.H.A.), and by a grant from USDA (D.W.).

307 The flowering-time gene FT and the floral transition

Takashi Araki, Hidetaka Kaya, Tomoshi Ohkawa, and Yasushi Kobayashi
Dept. of Botany, Grad. School of Science, Kyoto University, Kyoto, Japan

Flowering in *Arabidopsis* is promoted by several interacting pathways. A photoperiod-dependent pathway mediates signals from photoreceptors to a transcription factor gene *CONSTANS* (*CO*), which activates downstream genes such as *FT* and *LEAFY* (*LFY*). Photoperiod-independent pathways, mediated by genes such as *FCA*, also activate *FT* and *LFY*.

Simultaneous over-expression of *FT* and *LFY* causes conversion of the whole shoot into a single terminal flower with one or two bracts, suggesting that the combined activity of both genes strongly promotes flowering. These two genes act in parallel pathways. *FT* primarily affects floral transition, while *LFY* affects mainly floral fate specification of meristem and plays a crucial role in transcriptional regulation of organ identity genes. The pathway downstream of *FT*, however, remains to be elucidated. The late-flowering gene *FD* is a good candidate of genes acting downstream of *FT*. The *FD* activity seems to be required for the precocious-flowering phenotype of *35S::FT*. However, double mutant phenotype and genetic interaction with other genes suggested that *FD* is also involved in *FT*-independent pathway(s). *FD* locus is represented by single allele. New *fd* alleles, as well as other downstream genes, will be identified by suppressor screening of *35S::FT*. Several candidates are currently being analyzed.

FT is a member of a small gene family which includes *TERMINAL FLOWER1* (*TFL1*). It was suggested that *TFL1* regulates phase transition in general, delaying vegetative-to-reproductive transition and preventing apical meristem from progressing into flower phase. *FT* seems have opposite effects on phase transition; *FT* promotes transition, while *TFL1* delays it. Genetic interaction suggests that the balance between the two is an important factor in determining the timing of transition.

308 The FPF gene family and flowering time control in *Arabidopsis*

Roland Borner, Grit Kampmann, Klaus Apel and Siegbert Melzer
Institute of Plant Sciences, ETH Zürich, Universitätsstrasse 2, CH-8092 Zürich

The genetic basis of floral induction has been analysed by using flowering time mutants of *Arabidopsis thaliana*. Several mutants have been identified that are impaired in flowering time control. However, genes with a subtle or redundant function for floral induction can't be identified by mutational approaches. Therefore, a molecular approach was taken to identify genes that are expressed during the transition to flowering and that might be involved in flowering time control. One of the identified genes was *FPF1* (Flowering Promoting Factor 1). *FPF1* is mainly expressed in the peripheral zone of the apical meristem immediately after floral induction in *Arabidopsis* and is also expressed later in floral meristems. The gene encodes a 12.6 kDa protein which has no homology to any identified genes with known function. Constitutive expression of the gene under control of the CaMV 35S promoter causes early flowering under long and short day conditions. Two closely related genes, *FLP1* and *FLP2* (*FPF1*-Like Proteins), have been

identified that show 92% and 80% amino acid identity to the FPF1 protein. Constitutive expression of each gene causes earlier flowering under both long and short day conditions. However, the expression pattern of FLP1 and FLP2, which was defined by quantitative RT-PCR, was different to that of FPF1: FLP1 and FLP2 are not expressed in the apical meristem after floral induction. FLP1 is mainly expressed in roots and flowers, and at a lower level in leaves. FLP2 is only expressed in leaves and in some parts of the flowers, mainly in the sepals. The analysis of transgenic plants carrying FPF1-, FLP1- or FLP2-promoter-GUS-constructs supports the expression pattern obtained by quantitative RT-PCR. To further characterize the function of the three genes, a mutant screen of transposon tagged En-lines was performed. For all three genes, an En-line with a transposon-element within the coding region has been identified. These lines will be analysed for a mutant phenotype.

309 Identification of (Search for) FLOWERING LOCUS C-Interacting Proteins in *Arabidopsis thaliana*

Jason A. Bubier and Michael R. Schläppi

Marquette University P.O. Box 1881 Milwaukee, WI 53233

The transition of a flowering plant from the vegetative phase to the reproductive phase of development is a critical change that is based upon both genetic and environmental determinants. Molecular and genetic analysis have determined FLOWERING LOCUS C (FLC) to be an integral constituent of the regulatory pathway responsible for the maintenance of the vegetative growth phase and the inhibition of the transition to the reproductive state. The sequence of FLC indicates that it is a MADS box gene. MADS domain proteins have previously been implicated in the regulation of other major developmental events such as the regulation of floral organ and meristem identity. Plant MADS domain regulatory proteins contain a moderately conserved domain, designated the K domain, which exhibits a coiled-coil structure and has been shown to mediate protein-protein interactions. In vivo and in vitro studies of other *Arabidopsis* MADS domain containing proteins, such as AGAMOUS (AG) have indicated that the K domain is required for function. We hypothesize that the K domain of FLC interacts with other proteins necessary for FLC's function: the maintenance of the vegetative growth phase. To identify potential FLC-interacting proteins we are using bait fusion proteins containing the K domain and carboxy terminal region of FLC in a yeast two-hybrid screen. Determining what protein(s) FLC interacts with will further characterize the regulation of the transition from vegetative to reproductive growth in *Arabidopsis thaliana* and provide a greater understanding of FLC's central role in the maintenance of the vegetative growth phase.

310 Changes in amino acid content during floral induction in the leaf exudate collected from the long day plant *Arabidopsis thaliana*

Laurent Corbesier, Pierre Lejeune, Georges Bernier and Claire Périlleux

Department of Plant Biology, Laboratory of Physiology, University of Liège, Bld. du Rectorat B22, 4000 Liège, Belgium

Support for theories suggesting the importance of nutrition in the control of the flowering process was brought in recent years by genetic modifications of carbohydrate or nitrogen metabolism in various plants. However, these experiments are insufficient to decide

whether or not there is a precise change in the relative availabilities of C and N for the apex if flowering is to be promoted. Analysis of the carbohydrate levels of the phloem sap have been reported for several photoperiodic plants and, in all cases, an increase of carbohydrate level in the sap was observed as a result of floral induction. Curiously, the possible changes in amino acids in the phloem sap at floral transition were not investigated so far, despite they are the second prevalent compound of this sap. Our aim here was to provide data concerning their levels in the phloem exudate of *Arabidopsis thaliana* plants induced to flower by either a single long day (LD) or a single displaced short day (DSD). Combination of these data with those concerning carbohydrates allowed us to calculate the changes of the organic C:N ratio caused in the phloem sap by floral induction.

We observed that (i) the two inductive treatments induced an increased export of amino acids in the phloem sap at times compatible with the export from the leaves of the floral stimulus. In the LD system, glutamine was the amino acid responsible of the increase then in the DSD system, an increase of the asparagine level was observed. This correlates well with results showing that asparagine is the predominant amino acid exported from leaves of dark-grown or dark-adapted *A. thaliana* plants (Lam et al. 1995. *Plant Cell* 7, 887-898). (ii) At floral induction, the leaf exudate C:N ratio increased early showing that the C supply to the shoot apical meristem was more increased than the N supply.

ACKNOWLEDGEMENTS: L.C. is grateful to the F.N.R.S. for the award of a research fellowship. PAI P4/15, ULg (Fonds Spéciaux) and EC Contract BI04 CT97-2231

311 The role of gibberellin metabolism in the control of *Arabidopsis* flowering
Sven Eriksson, Thomas Moritz and Ove Nilsson
Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83, Umeå, SWEDEN

Gibberellins promote flowering of *Arabidopsis* by activating the LEAFY promoter. Under short day conditions, when gibberellins are absolutely required for flowering, LEAFY is expressed in young leaf primordia. The promoter activity gradually increases in these newly initiated primordia until the time of floral initiation when the fate of the primordia is shifted from a leaf/shoot fate to a flower fate. In a GA-mutant background, LEAFY promoter activity is abolished under short days. However, this activity (and flowering) can be restored by the application of gibberellins. These findings raise the question whether the gradual increase in LEAFY promoter activity found in the *Arabidopsis* shoot apex is correlated to a simultaneous increase in the levels of active gibberellins? Thus, a gradual increase in shoot apical gibberellin levels could be a way for the plant to monitor developmental age under non-inductive growth conditions. To investigate this, we have developed methods that enable the quantification of a wide variety of gibberellin species in milligram amounts of plant material. We have used this method, coupled to microdissections of plant material from the *Arabidopsis* shoot apex, to characterize changes in gibberellin metabolism during growth in short days in the very same tissues that express the LEAFY promoter. The results from these experiments will be presented. Furthermore, although it is now widely established that, in *Arabidopsis*, GA4 is the active gibberellin controlling elongation growth, there is still some controversy regarding which gibberellin is primarily active in the regulation of flowering.

In a complementary experiment, we are utilizing an *in vitro* assay to study the effects of various gibberellin species on the activity of the LEAFY promoter. These results, in combination with the quantifications described above, allow us to predict which gibberellin species that is the most likely to be the principal regulator of GA-controlled flowering in *Arabidopsis*.

312 EARLY IN SHORT DAYS (ESD1) a locus mediating short day inhibition of flowering in *Arabidopsis*

Gómez-Mena, C., Martín Trillo, M.M., Ruíz-García, L., Jarillo, J.A., Salinas, J., and Martínez-Zapater, J.M.

Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología, CSIC. Cantoblanco 28049, Madrid (Spain) and Departamento de Mejora Genética y Biotecnología, SGIT-INIA, Ctra. de La Coruña Km 7, 28040 Madrid (Spain)

Flowering in *Arabidopsis* is regulated by photoperiod, being accelerated under long days (LD) and inhibited under short days (SD). Several genes required for the long day induction of flowering have been identified, cloned, and placed in the so called LD pathway. However, information on the genes and pathways regulating flowering inhibition is still fragmentary and their interaction with the flowering induction pathways is not generally known. Two mutations at the ESD1 locus cause early flowering under non-inductive SD, suggesting that this locus is involved in the repression of flowering under those photoperiodic conditions. These mutations map on chromosome 3, close to the centromere position, and are not allelic of other early flowering mutations such as *hy2* or *pef1* also mapping on this chromosome. The early flowering phenotype of *esd1* mutants results from shortening of all the developmental phases of the plant, including the juvenile phase. These mutants also show a moderate elongation of hypocotyl and inflorescence internodes, leaf curling and extra organs in the flower perianth. The analysis of hypocotyl elongation of mutant seedlings under different monochromatic lights indicates that the mutants have a reduced sensitivity to red light. Furthermore, mutant plants grown under continuous red light flower earlier than wild type plants, which are strongly delayed under those conditions whereas no significant difference in flowering time is observed under continuous blue light. These results suggest the involvement of ESD1 in a phytochrome dependent pathway of flowering inhibition, what has been confirmed by the analyses of phytochrome deficient double mutants. Based on the characterization of double mutants carrying *esd1* and mutations in the different flowering inductive pathways we show that flowering inhibition mediated by ESD1 is not interacting with the autonomous or the LD inductive pathway but functions in parallel to them.

313 Genetic analysis of photoperiodic floral induction

Karen A. Hicks^{1,2}, Lydia M. Gordon¹, Adrienne D. Skrzypek¹, and D. Ry Wagner²
¹ Biology Department, Kenyon College, Gambier OH 43022; ² Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The timing of floral initiation is controlled in part by photoperiod, and ELF3 is required for proper daylength regulation of flowering. *elf3* null alleles cause photoperiod-insensitive early flowering, and also alter circadian regulation and photomorphogenesis.

The ELF3 gene has been cloned, and is predicted to encode a novel circadian-regulated protein that may function as a transcription factor.

In order to identify genes that function with or downstream of ELF3, we have isolated EMS-induced suppressors and enhancers of the *elf3-1* null allele by screening for alterations in hypocotyl length or flowering time. Thus far, 25 independent suppressor or enhancer mutants have been identified for further study. Eight recessive suppressor mutations either partially or fully restore photoperiod sensitive floral initiation in the absence of ELF3 function. One of these mutations also suppresses the photomorphogenesis defect of *elf3-1*. We will present our progress on the phenotypic and genetic analysis of these suppressor mutations, as well as our progress on mapping these mutations.

We have also initiated a screen for enhancers of the photoperiod-sensitive *elf3-7* weak allele. Second-site mutations which result in early flowering in short day conditions are being isolated in the *elf3-7* mutant background, and then rescreened in both long day and short day conditions to identify enhancer mutations which result in a loss of photoperiod sensitivity.

314 Identification of mutations which affect the flowering-time of FT over-expressors
Yasushi Kobayashi, Tomoshi Ohkawa, Hidetaka Kaya, and Takashi Araki
Department of Botany, Division of Biological Science, Graduate School of Science,
Kyoto University, Kyoto 606-8502, Japan

The flowering-time gene FT is an important positive regulator of floral transition. Molecular genetic interactions of FT with CO, TFL1, and LFY have been reported (1-3). FT acts in part downstream of CO, and in parallel with LFY (2, 3). Simultaneous over-expression of FT and LFY causes extremely precocious flowering, almost eliminating vegetative phase. This suggests that the combined activity of FT and LFY is a limiting factor for flowering. Therefore, to understand the floral transition process, it is important to identify the downstream components of FT and LFY pathways. Downstream genes in the LFY pathway for floral fate specification and patterning were extensively analyzed (4, 5). In contrast, genes acting downstream of FT to eventually cause floral transition have not yet been identified. To elucidate components of the FT pathway, we have taken two complementary approaches.

One approach is to test the effect of known flowering-time mutations on the precocious-flowering phenotype in 35S::FT lines. We found that the late-flowering mutation *fd-1* partially suppresses early-flowering phenotype of 35S::FT, whereas others do not. Effect of *fd-1* can be interpreted rather strong, given its rather weak phenotype in non-transgenic background.

The other is systematic screening of suppressor mutants of 35S::FT lines. We mutagenized a weak and a strong lines of 35S::FT, and performed family screening for both of them. So far, we have obtained several candidates from both lines.

Characterization and allelism test of these suppressor candidates with *fd-1* are currently in progress.

One enhancer candidate was also obtained during the screening. It exhibits extremely precocious-flowering phenotype, with no true leaves. *tfl1* mutations were shown to enhance 35S::FT phenotype (2, 3). However, the phenotype of a new enhancer is clearly different from that of 35S::FT; *tfl1* or other mutants such as *emf*.

- (1) Luiz-Garcia et al. *Plant Cell* 9, 1921-1934 (1997).
- (2) Kobayashi et al. *Science* 286, 1960-1962 (1999).
- (3) Kardailsky et al. *Science* 286, 1962-1965 (1999).
- (4) Parcy et al. *Nature* 395, 561-566 (1998).
- (5) Wagner et al. *Science* 285, 582-584 (1999).

315 Induction of ectopic floral meristems on leaves of *fil/yabby3* double mutants in *Arabidopsis*

Mande Kumaran and Venkatesan Sundaresan

Institute of Molecular Agrobiology, 1, Research Link, National University of Singapore

We had previously reported the molecular cloning of ABNORMAL FLORAL ORGANS gene which is required for floral meristem maintenance and flowers formation (1). The AFO gene is identical to ANTHERLESS (1), FILAMENTOUS FLOWER (2) and YABBY1, a member of YABBY gene family of putative transcription factors (3). Overexpression of two of the YABBY family members called FIL and YABBY3 suggests that these genes promote abaxial cell types identity in lateral organs which are produced post-embryonically from shoot apical meristem (2,3). Although the FIL expression pattern is observed abaxially in both vegetative and floral development, *fil* mutants exhibit only floral defects, possibly due to redundancy with YABBY2 and YABBY3 (3). To address the function of FIL/YABBY in vegetative development, we have isolated a *yabby3* mutant allele using Ds gene trap insertional mutagenesis. We generated double mutant for *fil* and *yabby3* for phenotypic analysis. These double mutant exhibits much stronger floral defects than the *fil* single mutant. Plants grow dwarf and bushy, leaves are serrated and narrower having very long petiole that can exhibit leaf cell types, suggesting that it is derived from the partial radialization of leaves. Unexpectedly, in some mutant plants, ectopic vegetative and floral meristems originated from the leaf surface. The occurrence of these meristems is more frequent at the base of the leaf margin, and is correlated with the region where the YABBY3 GUS reporter gene shows high level of expression. It appears that in the absence of FIL and YABBY3, the cells of the marginal meristem of the leaf can revert to shoot apical meristems. It has been shown that KNAT1 overexpressing transgenic lines produce ectopic meristem on leaves (4). Moreover, the ectopic expression of FIL/YABBY3 suppresses maintenance of the SAM (2,3) which requires expression of knotted-like genes. We speculate that in WT leaf development, FIL and YABBY3 may act either directly or indirectly to down-regulate the knotted-like genes of *Arabidopsis*. References 1. Kumaran M.K et.al (1999) *Sex plant Reprod.* 12, 118-122. 2. Sawa et.al (1999) *Genes Dev.* 13, 1079-1088. 3. Siegfried et al (1999) *Development.* 126, 4117-4128. 4. Chuck G et.al (1996) *Plant Cell.* 8, 1277-1289.

316 The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*

Ilha Lee¹, Horim Lee¹, Sung-Suk Suh¹, Eunsook Park¹, Euna Cho¹, Ji Hoon Ahn²
¹ School of Biological Sciences and Research Center for Cell Differentiation, Seoul National University, Seoul, 151-742, Korea; ² Plant Biology Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, C

The very late-flowering behavior of *Arabidopsis* winter-annual ecotypes is conferred mainly by two genes, FRIGIDA (FRI) and FLOWERING LOCUS C (FLC). A MADS-domain gene, AGAMOUS-LIKE 20 (AGL20), was identified as a FRI suppressor in activation tagging mutagenesis. Overexpression of AGL20 suppresses not only the late flowering of plants that have functional FRI and FLC alleles, but it also suppresses the delayed phase transitions during the vegetative stages of plant development. Interestingly, AGL20 expression is not only positively regulated by the redundant vernalization and autonomous pathways of flowering, but also by the photoperiod pathway. Our results indicate that AGL20 is an important integrator of three pathways controlling flowering in *Arabidopsis*.

317 ELF3 encodes a circadian-regulated nuclear protein that functions in an *Arabidopsis* PHYB signal transduction pathway

Xing Liang Liu^{1,4}, Michael F. Covington^{1,5}, Christian Fankhauser^{2,6}, Joanne Chory^{2,3}, and D. Ry Wagner^{1,4}

¹Institute of Molecular Biology, University of Oregon, Eugene, OR 97403, USA. ²Plant Biology Laboratory and ³Howard Hughes Medical Institute, Salk Institute, La Jolla, CA 92037, USA

Many aspects of plant development are regulated by photoreceptor function and the circadian clock. Loss-of-function mutations in the *Arabidopsis* EARLY FLOWERING 3 (ELF3) and PHYTOCHROME B (PHYB) genes cause early flowering and influence the activity of circadian-regulated processes. We demonstrate here that the relative abundance of ELF3 protein, which is a novel nuclear-localized protein, displays circadian regulation that follows the pattern of circadian accumulation of ELF3 transcript. ELF3 interacts with PHYB in the yeast two-hybrid assay and *in vitro*. Genetic analyses show that ELF3 requires PHYB function in early morphogenesis but not for the regulation of flowering, while PHYB requires ELF3 function for the control of both early morphogenesis and floral initiation. This suggests that ELF3 may interact with other photoreceptors to regulate the photoperiodic induction of flowering in *Arabidopsis*.

⁴ Present Address: Agritope, Inc., 16160 SW Upper Boones Ferry Road, Portland, OR 97224, USA.

⁵ Present Address: Department of Cell Biology, The Scripps Research Institute, La Jolla, CA, 92037, USA.

⁶ Present Address: Département de Biologie Moléculaire, Université de Genève, 1211 Genève 4, Switzerland.

318 Molecular and genetic analyses of the cold input to vernalization

Jiayou Liu, Sarah J. Gilmour, Michael F. Thomashow, and Steve van Nocker

Department of Horticulture, Michigan State University, East Lansing, MI 48824

The acceleration or induction of flowering by prolonged exposures to near-freezing temperatures, a process known as vernalization, has been well-studied only at the physiological level, and the molecular components of the cold-sensing mechanism remain unknown. In genotypes of *Arabidopsis* that have a winter-annual flowering habit, the end effect of prolonged cold exposure is the transcriptional repression of the

flowering-inhibitor gene *FLC*. We are interested in events that occur upstream of *FLC* repression. We examined the potential involvement of two factors that are known to play a role in cold exposure, (1) *CBF1*, an AP2-domain-type transcriptional regulator that acts to induce the expression of a suite of cold-responsive (*COR*) genes, and (2) the phytohormone abscissic acid (*ABA*), whose levels increase during cold exposure. We introduced a transgene driving constitutive expression of *CBF1* into a winter-annual genotype of *Arabidopsis*. This was sufficient to induce constitutive, high-level expression of the *CBF1* regulon of *COR* genes, but had no promotive effect on flowering time in the absence of cold exposure. We also introduced various mutations that compromise *ABA* biosynthesis or response into a winter-annual genotype, and found that the resulting genotypes exhibited a vernalization response that was indistinguishable from that of the wild-type, winter annual parent. Taken together, these findings indicate that vernalization involves factors distinct from *ABA*, *CBF1*, and the *CBF1* regulon of *COR* genes. Using microarray technology and complementary approaches, we have identified several genes whose mRNA levels increase or diminish significantly after extended exposures to cold, and thus are potential components of the flowering-associated cold-sensing mechanism.

319 A first glimpse into the molecular nature of vernalization: epigenetic regulation of *FLC* expression

Scott D. Michaels and Richard M. Amasino

University of Wisconsin-Madison, Department of Biochemistry, 433 Babcock Drive, Madison, WI 53706

The promotion of flowering in response to a prolonged exposure to cold temperatures (i.e. winter) is a useful adaptation for plant species that flower in the spring. This promotion is known as vernalization. While the physiology of vernalization has been extensively studied in many species, the molecular mechanism of vernalization remains largely unknown. In *Arabidopsis* naturally occurring late-flowering ecotypes and plants containing late-flowering mutations in the autonomous floral-promotion pathway are relatively late flowering unless cold treated. The vernalization requirement of these late flowering ecotypes and autonomous pathway mutants is largely created by an upregulation of the floral inhibitor *FLOWERING LOCUS C (FLC)* in these backgrounds. After cold treatment, as imbibed seeds or young seedlings, *FLC* transcript levels are downregulated and remain low for the remainder of the plant's life, but return to high levels in the next generation. Plants containing a constitutively expressed 35S:*FLC* construct remain late flowering after cold treatment, indicating that *FLC* levels must be downregulated for vernalization to be effective. Thus the epigenetic downregulation of *FLC* appears to be a major target of the vernalization pathway and provides the first molecular marker of the vernalized state.

320 *FKF1*, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*

David C. Nelson, Jamie Lasswell, Luise E. Rogg, Mindy A. Cohen, and Bonnie Bartel
Department of Biochemistry and Cell Biology, Rice University, 6100 S. Main, Houston, Texas, 77005

Successful plant reproduction requires precise control of the transition to flowering in response to environmental cues. We have isolated an Arabidopsis mutant, *fkf1*, with a late flowering phenotype in long days that is rescued by vernalization or gibberellin treatment. The mutant also exhibits a light-dependent hypocotyl elongation defect. We used a positional approach to clone FKF1 (flavin-binding, kelch repeat, F-box), which encodes a novel protein with an N-terminal PAS domain similar to the flavin-binding region of certain photoreceptors, an F-box motif characteristic of proteins that target specific substrates for ubiquitin-mediated degradation, and six kelch repeats predicted to fold into a γ -propeller and mediate protein-protein interactions. There are two close homologs of FKF1 in Arabidopsis, ZTL and FKL1. Constitutive expression of ZTL induces hypocotyl elongation in the light and lengthens flowering time. FKF1 is highly expressed in leaves, and is also expressed in anther filaments, stomata, sepals, and root tips. FKF1 mRNA levels oscillate with a circadian rhythm and deletion of FKF1 alters the waveform of rhythmic expression of two clock-controlled genes, implicating FKF1 in modulating the Arabidopsis circadian clock.

321 Assignment of circadian function for the Arabidopsis clock related gene GI
Deok Hoon Park, David E. Somers¹, Hyo Jung Kim, Don Ha Park, Steve A. Kay¹ and Hong Gil Nam

Dept. of Life Science, Pohang University of Science and Technology, Pohang, Korea, ¹ Dept. of Cell Biology, The Scripps Research Institute, CA 92307, USA

Photoperiodic responses in plants such as day-length dependent flowering are critical for adaptive developmental decisions. We report that mutations in the Arabidopsis GI gene conferring photoperiodic-insensitive flowering cause global alteration of circadian rhythms. Cyclic expression of the GI gene and the putative clock-associated genes, LHY and CCA1, shows a significantly altered period length, reduced amplitude and more rapid damping in the *gi* mutants. Thus, GI is required for maintaining circadian amplitude and proper period length of these genes. Also clock-regulated genes including CAT2, CAT3, AtGER3 and AtGRP7 showed altered amplitude and phase in constant light or dark after entrainment. We are studying the relationship between other environmental factors and the *gi* mutants.

322 Search for functional homologs of FLOWERING LOCUS C in biennial plants

Monica Patel, Ying Pan, Michael Schläppi

Marquette University

The MADS box gene FLOWERING LOCUS C is a central regulator of floral repression in Arabidopsis thaliana. Overexpression of FLC mRNA causes late flowering, unless the plants are vernalized. Transgenic Arabidopsis plants will senesce without flowering if FLC transcript levels are very high. However, naturally occurring late flowering ecotypes of Arabidopsis do flower eventually without vernalization. By contrast, biennial plants such as Beta vulgaris (sugar beet) and Hyoscyamus niger (henbane) do not flower unless they are vernalized. We hypothesize that high transcript levels of FLC homologs repress flowering in biennial plants and may thus be responsible for biennialism. Using three molecular methods, we are presenting and discussing progress towards the identification of a potential FLC homolog in biennial henbane. FLC probes were used first in Southern and second in Northern hybridization experiments to determine whether sequence

conservation exists between *Arabidopsis* and biennial henbane. In a third set of experiments, RT-PCR were carried out to isolate cDNA of potential FLC homologs. Since it is possible that functional, but not structural, homology is conserved for FLC between *Arabidopsis* and biennial plants, we are also isolating MADS box genes that are exclusively expressed in biennial, but not annual, henbane. Such genes are additional candidates for functional homologs of floral repressors in a biennial plant.

323 The late-flowering, aerial rosette-bearing phenotype of the Sy-0 ecotype requires a synergistic interaction of dominant alleles of at least three genes: FRI, ART1 and ART2
Antje Redweik and Vojislava Grbic
Department of Plant Sciences, University of Western Ontario, London, ON, Canada
We have identified a new late flowering locus ART1 that maps on chromosome 5, between the ms1 and ttg morphological markers in a genomic interval defined by the MYJ24 and MKD15 BACs. We will report on the progress of cloning the ART1 locus by positional cloning. In a process of fine mapping of ART1, we have determined that the dominant allele of ART1 has no phenotype of its own. ART1 requires the presence of a dominant allele of ART2 to confer a late flowering phenotype. ART2 is linked to ART1 and maps upstream of the lu marker. Currently we are testing the possibility that ART2 is an allele of FLC.

324 FLC protein acts as a repressor of flowering in *Arabidopsis*
Dean T. Rouse, Candice C. Sheldon, David J. Bagnall, W.J. Peacock and Elizabeth S. Dennis
CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2611 Australia
The FLC protein predicted from a knowledge of the sequence of the FLC gene belongs to the MADS box class of protein and is thought to function as a transcription factor. We have raised antibodies to bacterially expressed FLC protein and examined the level of FLC protein in different ecotypes and mutants. Late flowering ecotypes and the late flowering overexpression mutant flc-11 have a high level of FLC protein whereas early flowering ecotypes and the early flowering loss of function mutant flc-13 have little or no FLC protein. FLC protein levels are up regulated in late flowering vernalization responsive mutants such as fca, fld and ld but not in the non vernalization responsive late flowering mutants such as co and gi. These results are consistent with the wild type FCA, FLD and LD acting to repress the FLC gene. Late flowering is associated with increased FLC protein levels with flowering time proportional to the level of FLC protein. The level of FLC protein is dramatically decreased following vernalization. Tissue distribution of FLC has also been examined. FLC protein is present in all tissues of the late flowering ecotypes and mutants with the highest level occurring in the shoot apex. The level of FLC protein mirrors that of the FLC transcript. Current research focusses on the method of down regulation of the FLC protein during vernalization and the targets of FLC action.

325 *Arabidopsis* flowering-time genes regulated by diverse genetic pathways are directly activated by CONSTANS
Alon Samach^{1,4}, Hitoshi Onouchi¹, Scott E. Gold², Gary S. Ditta², Zsuzsanna Schwarz-Sommer³, Martin F. Yanofsky² and George Coupland¹

1John Innes Centre, Norwich,UK. 2Dept.of Biology,UCSD,CA,USA.3Max Planck Institut fuer Zuechtungsforschung,Koeln,Germany.4Horticulture Dept.Hebrew University of Jerusalem,Rehovot,Israel

In plants, the transition to flowering is triggered by endogenous and environmental signals. Genes that regulate this transition in *Arabidopsis* have been assigned to separate genetic pathways that control the response to stimuli. These pathways must converge to regulate expression of genes involved in floral development, but how this occurs is unknown. *CONSTANS* (*CO*) promotes flowering in response to daylength. Transgenic *Arabidopsis* plants in which the *CO* gene is over expressed from the 35S viral promoter (35S:*CO*) flower early in both long and short days, and are insensitive to daylength. We chose to identify the genes *CO* activates, determine whether other flowering pathways control them, and examine their role in flowering. Two complementary approaches, a screen for mutations in genes that suppress the 35S:*CO* phenotype, and a screen for genes that are directly activated by *CO*, were used. Several genes with diverse functions were identified and shown to be required for *CO* to promote flowering. Expression of these genes is also controlled by a second pathway associated with the control of flowering in response to low temperature, and by age. Thus flowering-time genes directly activated by *CO* identify points of convergence of pathways that respond to different internal and environmental stimuli.

326 Col-0/No-0 inbred line 114-1-1 of *Arabidopsis thaliana* contains a weak allele of *FLOWERING LOCUS C*

Michael Schläppi

Marquette University, 530 N. 15th St., Milwaukee, WI 53233

Arabidopsis plants are late flowering when the MADS box gene *FLOWERING LOCUS C* (*FLC*) is expressed above a certain threshold. *FLC* is positively and negatively regulated by multiple inputs. Vernalization decreases the abundance of *FLC* mRNA. It is shown here that there is a linear relationship between the length of vernalization and the resulting reduction of flowering time and the abundance of *FLC* mRNA. Conversely, dominant alleles of *FRIGIDA* (*FRI*) and recessive mutants of flowering time genes such as *FCA* and *LD* increase the abundance of *FLC* mRNA. *FRI* particularly upregulates strong alleles of *FLC*, which are found in most *Arabidopsis* ecotypes. However, weak alleles of *FLC* that are only partially activated by *FRI* were found in two ecotypes, *Ler* and *C24*. Here it is shown that a *No-0* allele of *FLC* is also weak in a particular inbred line of *Col-0/No-0*. The new allele was uncovered in a *Ds* tagging screen as the relatively early flowering line 114-1-1. The recombinant *Col-0/No-0* background and not the transposed *Ds* element correlated with the early flowering phenotype, because neither a mutation nor a *Ds* insertion were found in the cDNA or the genomic DNA of the 114-1-1 allele, respectively. Genetic crosses of a line containing *FRI* and a strong allele of *FLC* with 114-1-1 indicated additive gene action and suggested that at least 2 to 3 interacting genes regulate flowering time in this background. Interestingly, some *Col-0/No-0* inbred lines lacking *FRI* were also relatively late flowering, which correlated with increased levels of *FLC* mRNA. By contrast, some *Col-0/Ler* inbred lines were uncovered that completely suppressed the *FRI*-mediated activation of *FLC*. Such lines were as early flowering as wild type *Ler*, but still contained the dominant allele of *FRI*. These data emphasize the central role of *FLC* for repression of flowering and that its transcriptional

and/or posttranscriptional regulation determines flowering time. The data also suggest that several quantitative trait loci (QTL) negatively and positively regulate the abundance of FLC mRNA. These QTL are from natural populations of Arabidopsis and are different from FRI and recessive mutants of flowering time genes.

327 PATTERN OF EXPRESSION OF CONSTANS PROTEIN

Federico Valverde, Paula Suarez-Lopez, Manuela Costa and George Coupland
Department of Molecular Genetics, John Innes Centre, Norwich, UK

In wild-type plants CONSTANS (CO) promotes flowering under long days (LD), so that co mutants flower later under LDs than wild type. Over-expression of CO in 35S::CO plants causes early flowering under both long and short days. CO protein belongs to a new family of plant transcriptional activators with a B-box motif at the amino-terminus, an acidic region in the middle of the protein and a conserved carboxy-terminal domain that includes a nuclear localisation signal. A CO-GFP fusion protein expressed from the 35S promoter drives early flowering, and by the use of fluorescence microscopy we have demonstrated that CO:GFP is present in the nuclei of these plants. GFP polyclonal antibodies are also being used to detect the CO-GFP fusion protein in plants by western blot. RT-PCR experiments have shown that CO mRNA shows a circadian regulated pattern of expression with a peak in mRNA abundance during the night, which differs in plants growing under short and long days. We have also overexpressed CO in E.coli with a His-tag amino-terminal addition, which has allowed purification of the His-tagged protein in cobalt affinity columns. His-CO protein has been injected in rabbits to obtain polyclonal antibodies that were used to detect CO protein both in the overproducing E.coli cells and plants. The antibodies are being used to test whether the CO protein shows a similar pattern of circadian clock regulated expression to the CO RNA.

328 SPLAYED, a SWI/SNF ATPase Homolog Controls Reproductive Development in Arabidopsis

Doris Wagner and Elliot M. Meyerowitz
California Institute of Technology

When an organism switches from one developmental program to another, complex regulatory circuits are required to ensure temporal and spatial coordination of the required cell fate changes. We have identified a key component for the temporal control of the switch from vegetative to reproductive development in Arabidopsis thaliana. The SPLAYED gene product appears to regulate shoot apical meristem identity by altering the activity of the LEAFY transcription factor in response to environmental stimuli. In addition, SPLAYED acts redundantly with LEAFY in regulation of floral homeotic gene expression. SPLAYED encodes a novel Snf2p ATPase homolog implicated in chromatin remodeling. That SPLAYED regulates flowering in response to environmental stimuli suggests that the profound effect of environmental cues on plant development may be achieved by regulating transcription factor activity via alteration of chromatin state.

329 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 Physiological Botany, Uppsala University

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 α -helices. About 20 ATHB genes have been isolated and they are highly conserved within the homeodomain region. Our aim is to determine the functions of the ATHB genes in *Arabidopsis*. Here we present the analysis and characterisation 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 flowering time as compared to wild type. (A) Rosette leaf expansion Overexpression of ATHB16 caused a reduction in leaf expansion and internode elongation, resulting mainly from a reduced expansion and 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 cell expansion, but not by an increase in cell numbers. The leaf length-to-width ratio is not altered in the transgenic plants. We propose that the ATHB16 gene product act 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. No effect on flower morphology was observed. We conclude from these results that ATHB16 may function as a negative regulator of the flowering time response to photoperiod. Recently, significantly higher ATHB16 expression level has been found in *fha* mutants, which is defective in the blue-light photoreceptor pathway.

330 A gene Expression Screen Identifies Earli 1 as a Novel Vernalization-Responsive Gene in *Arabidopsis Thaliana*

Richard Wilkosz and Michael Schlappi

Department of Biology, Marquette University

Vernalization promotes early flowering in late ecotypes of *Arabidopsis thaliana*, however the mechanisms of vernalization are poorly understood. A subtractive hybridization approach was used to isolate vernalization-responsive genes from a late flowering ecotype of *Arabidopsis thaliana* based on the premise that transcript levels of such genes would increase with cold treatment and remain high even after removal of the vernalization stimulus. As a result, a gene fragment corresponding to the EARLI 1 gene was isolated. The abundance of EARLI 1 RNA is progressively elevated by vernalization and remains high for at least 20 days at room temperature. The basal level of EARLI 1 RNA is higher and is also increased after vernalization in early flowering ecotypes. Vernalization and subsequent growth in long-day photoperiods has an additive or synergistic effect on EARLI 1 activation. EARLI 1 RNA levels are also transiently induced by cold shock and jasmonic acid, but not by abscisic acid. Expression levels in response to cold shock quickly dissipate within 24 hours. EARLI 1 is thus a novel vernalization-responsive gene in *Arabidopsis thaliana* that can be used to investigate vernalization-specific transcriptional regulation.

331 Isolation and characterization of activation-tagged Arabidopsis mutants sls1 and sls2 suppressing late-flowering phenotype under short day

Naoto YABE, Lisa SHOJI and Kohji HASUNUMA

Division of Cytogenetics, Kihara Institute for Biological Research, Yokohama City University

Arabidopsis is a facultative long-day plant showing late-flowering phenotype under short-day condition. To identify genes regulating transition from vegetative to reproductive growth, we tried to isolate activation-tagged early flowering mutants under short-day condition. We have established more than 10,000 independent activation-tagged lines using Columbia ecotype. Screening was performed growing hygromycin resistant T1 plants under continuous illumination and T2 plants under short-day (8L/16D) condition, and picking up candidates that bolt their inflorescences earlier than wild type by date and rosette leaf number.

We have isolated 2 dominant mutants designated as sls1 and sls2 (suppress late-flowering phenotype under short day). sls1 opens its first flower at the stage when rosette leaf number comes to 14.1 ± 0.5 under short-day condition, when non-transgenic Columbia plants grown under the same condition have more than 40 rosette leaves. Under long-day condition, sls1 also flowers early at the stage of 5 ± 0.5 rosette leaves. sls1 shows other visible phenotypes than early flowering as follows; 1. young rosette leaves curl upward like curly leaf mutant, but mature rosette and cauline leaves are normal. 2. semi-dwarf phenotype by partially lacking secondary metamer.

sls2 shows early flowering phenotype both under short-day and long-day condition, but first flower opens late after bolting when grown under short-day, showing elongated internode phenotype in its rosette stage.

We have obtained mutants carrying single T-DNA insertion from backcrossed segregants, and isolated flanking genomic sequence by plasmid rescue method. T-DNA carried by sls1 mutant inserted in the putative spacer region on chr.III. No flowering-related gene was existed around the insertion site suggesting that it might be a novel factor positively regulate transition from vegetative to reproductive phase. To identify the gene causing early flowering phenotype, Northern analysis and transformation experiments are now underway.

332 Functional Analysis of EMBRYONIC FLOWER 2 Gene in Arabidopsis

Nobumasa Yoshida, Yukihiro Yanai, 1Lingjing Chen, 1Z. Renee Sung, and Shigeru Takahashi

Life Science Laboratory, Mitsui Chemicals, Inc., 1Plant and Microbial Biology, UC Berkeley

Mutations in EMBRYONIC FLOWER genes (EMF1,EMF2) cause a bypassing of the vegetative phase leading to extremely early flowering in Arabidopsis. This mutant phenotype suggests that EMF activities play a major role in floral transition. We have isolated EMF2 by map-based cloning and revealed the gene encodes a novel zinc finger protein1). EMF2 is a member of a small multigene family in Arabidopsis and shares partial homology with Fertilization Independent Seed 2 (FIS2). In situ RNA hybridization demonstrated that EMF2 expression can be detected in all major developmental stages of Arabidopsis. In embryogenesis, the transcripts were observable in whole embryo and

endosperm. After germination, the transcripts were specifically accumulated in both vegetative and reproductive meristems. In addition, Promoter::GUS analysis showed EMF2 expressed in root meristems as well as reproductive organs such as anthers and pistils. These results suggest that EMF2 functions as a developmental regulator throughout the life cycle of Arabidopsis. Introduction of 35S::EMF2 constructs into wild type plants generated no late-flowering plants but early-flowering plants bearing terminal flowers, possibly caused by co-suppression. The involvement of EMF2 in floral transition will be discussed.

1) Yoshida, N. et al. (1999) Abstract in the 16th International Botanical Congress.

333 NOZZLE couples pattern formation and growth during floral organogenesis
Sureshkumar Balasubramanian and Kay Schneitz*

Institute of Plant Biology/University of Zurich, 107, Zollikerstrasse, Zurich, Switzerland.

*email: Kay.Schneitz@access.unizh.ch

Pattern formation, growth and morphogenesis are distinct, yet coupled processes that occur repeatedly during development. We are interested in studying the molecular genetic basis of these processes using the ovules of Arabidopsis as the model system. Ovules of seed plants have a stereotypic arrangement of pattern elements along the proximal-distal axis with the nucellus at the distal end, chalaza in the center and the funiculus at the proximal end. It has been proposed that this pattern be set up during the early proliferative phase of ovule development. What is the underlying mechanism and how is it orchestrated with cell proliferation? BEL1 and more recently NOZZLE have been implicated to play a role in this process. NZZ encodes a novel protein localized to the nucleus (1). Here we show that NZZ plays a central role in pattern formation, and it couples this process with growth. We present our data, which suggest that NZZ is a transcriptional repressor. NZZ plays a major role in the formation of the nucellus by negatively interacting with AINTEGUMENTA a gene involved in cell proliferation, BEL1 which plays a role in proximal-distal pattern formation and INNER NO OUTER which is involved in the abaxial-adaxial patterning in the ovules. In part this is achieved by repressing the BEL1 and ANT mRNA in the nucellus. Furthermore, NZZ acts redundantly along with BEL in specifying chalaza. Plants defective for nzz and bel carry ovules that show a "pattern duplication" because the chalaza is replaced with funicular tissue. NZZ participates in the growth of the funiculus again through its negative interactions with ANT & INO. NZZ also regulates the growth of the developing integuments and here it represents a target gene for the putative transcription factors ANT and INO. In summary, NZZ interacts with BEL & INO, which are patterning genes, and with ANT which is involved in growth control. Therefore, NZZ appears to couple these distinct but intimately linked processes.

1. Schiefthaler, U et al, (1999) Molecular analysis of NOZZLE, a gene involved in pattern formation and early sporogenesis during sex organ development in Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA 96: 11664-11669.

334 Genetic analysis of larsen, an enhancer of leunig and leunig-like in flower development

Xiaozhong Bao¹, Robert G. Franks¹, Joshua Z. Levin², and Zhongchi Liu¹

¹Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742; ²Novartis Agribusiness Biotech Research, Inc., Research Triangle Park, NC

The LEUNIG (LUG) and LEUNIG-LIKE (LGL) genes are required for the repression of AGAMOUS (AG) during flower development. *lug* and *lgl* mutants display ectopic expression of AG in the first two whorls of a flower, resulting in homeotic conversions of floral organ identities and a reduction of floral organ number. Genetic interactions between *lug* and *lgl* and among *lug*, *lgl*, *ag*, *ant*, and *ap2* suggested that the functions of LUG and LGL are closely related and partially redundant. *larsen* (*lsn*), a novel mutant without any obvious phenotype by itself, drastically enhances *lug* and *lgl* mutants. Both *lsn lug* and *lsn lgl* double mutants show severe reduction in the size of 4th whorl carpels, and exhibit more carpelloid or staminoid 1st whorl organs. However, the enhancement of *lug* by *lsn* appears stronger than the enhancement of *lgl* by *lsn*. These analyses suggest that LSN acts in the same pathway as LUG, but its function is redundant with LUG. Thus LSN's function can only be revealed in the absence of LUG. LSN maps to chromosome V between PDC3 and CTR1. Since no other mutation with similar properties to *lsn* is located in this region, *lsn* likely defines a new gene for the regulation of flower development.

335 Role of CLAVATA 3 in Regulating Stem Cell Fate in Arabidopsis

Ulrike Brand¹, Jennifer C. Fletcher^{2,3}, Martin Hobe¹, Elliot M. Meyerowitz³ and Rüdiger Simon¹

¹Universität zu Köln, Gyrhofstr. 17, 50923 Köln, Germany; ²PGEC, Agriculture Research Service, 800 Buchanan St., Albany, CA 94710, USA; ³CalTech, 1200 East California Boulevard, Pasadena, CA 91125, USA

In higher plants, organogenesis occurs continuously via initiation of primordia from self-renewing apical meristems. Mutations in *clavata* 1, 2 or 3 cause an increase in meristem size and the formation of fasciated stems and production of extra floral organs. Genetic analysis indicated that the CLV genes interact to control the balance between meristem cell proliferation and differentiation. The CLV1 and CLV2 genes encode LRR-transmembrane proteins, only the CLV1 protein carries a kinase domain. These two proteins are assumed to form a membrane bound receptor protein complex, together with a kinase associated phosphatase (KAPP) and a Rho-GTPase related protein. The third gene, CLV3, encodes a small, predicted extracellular protein that is required for the formation of an active complex. We examined whether stem cell accumulation and fate in shoot and floral meristems depends directly on the level of CLV3 activity by expressing CLV3 under control of the CaMV35S promoter. The transgenic plants resembled wild-type at early stages of development, but ceased to initiate organs after formation of the first leaves. Flower meristems that were produced during later stages failed to initiate the inner whorl of organs, indicating that stem cells were not correctly specified and/or maintained when CLV3 was expressed constitutively at high levels. Phenotypically, these plants resembled *wuschel*-mutants. RNA in situ hybridisation experiments revealed that expression of WUS is reduced in the transgenic lines. When the 35S::CLV3 transgene was introduced into *clv1* or *clv2* mutants, all plants exhibited the typical *clv* mutant phenotype, indicating that CLV3 signalling requires functional CLV1 and CLV2. We

conclude that the CLV1, 2 and 3 act together in a signal transduction complex that negatively regulates stem cell fate by repressing the activity of wus. WUS has a role in a stem cell promoting pathway, since it is required for the induction and maintenance of stem cells that express the CLV genes.

336 Isolation and Characterization of Reproductive Actin Mutations in *Arabidopsis thaliana*

Lucia Cardenas Pawloski, Elizabeth McKinney, and Richard B. Meagher
University of Georgia, Athens, GA 30602-7223

Actin is an essential cytoskeletal protein that is involved in several critical subcellular processes in plants. The *Arabidopsis thaliana* actin family has eight expressed genes that have been conserved for 300-450 million years. These genes are divided into two classes, vegetative or reproductive. The reproductive actin genes are predominantly expressed in developing and reproductive tissues, such as pollen, pollen tubes, ovules, and developing seeds. A better understanding of the selective constraints acting to preserve this actin class can be obtained by examining mutants. Using PCR to look for T-DNA insertions in actin genes, we screened 60,000 mutagenized *Arabidopsis* lines from the University of Wisconsin collection. Using degenerate primers, numerous putative positive signals were identified. Based on sequence of the T-DNA junctions from a few of these signals, we have already found mutations in five actin genes. Combined with previously identified mutants, we now have mutants in six actin genes and multiple mutations in three. Mutations have been found in three reproductive actin genes: act3-2, act3-3, act4-1, and act11-1. To reveal phenotypes for each reproductive actin mutation, we will perform pollen competition assays, analyze mRNA and protein expression levels, grow plants under various stress conditions, and perform multigenerational population studies. By characterizing these mutations, we will have a better understanding of the importance of the reproductive class of actins in all higher plants.

337 EMF1 Specifies Phase Changes and Shoot Architecture in *Arabidopsis*

Lingjing Chen, Dominique Aubert, Yong-Hwan Moon, and Z. Renee Sung
Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

Arabidopsis shoot undergoes phase changes during growth and development. The interplay of a large number of flower promotion and flower inhibition genes specifies the length and type of developmental phases. Embryonic Flower (EMF) genes, EMF1 and EMF2, are involved in every phase of development by promoting the vegetative and repressing the reproductive development. We cloned the EMF1 gene through a map-based strategy. The EMF1 gene encodes a 121-kDa protein with no similarity to any known proteins. Nevertheless, several domains such as nuclear localization signals, LXXLL motif, homopolymeric regions of serine, putative phosphorylation sites, and an ATP/GTP binding motif (P-loop) could be identified. EMF1 is expressed in roots, rosette leaves, stems, cauline leaves and flowers. The transgenic plants harboring a sense or an antisense EMF1 construct under the control of the CaMV 35S promoter display a spectrum of phenotypes that show a progressive change in shoot determinacy, meristem identity, and metamer types. The series of 35S::EMF1 plant phenotypes supports the

Controller of Phase Switching (COPS) hypothesis (Schultz and Haughn, 1993) that a continuous process specified by a gradient of EMF1 activities underlies phase transitions, which in turn determine shoot morphology and architecture.

338 Beyond the ABC model: regulators of stamen and carpel identities in *Arabidopsis*
Xuemei Chen, Tamara Western, and Yulan Cheng

Waksman Institute, Rutgers, the State University of New Jersey, Piscataway, NJ 08854

We are interested in the developmental mechanisms that underlie the formation of stamens and carpels from undifferentiated cells in the floral primordium in *Arabidopsis thaliana*. Although several MADS domain proteins, such as AGAMOUS (AG), APETALA3 and PISTILLATA, are known to serve as master regulators in the specification of stamen and carpel identities, it is not known how the actions of these master regulators are translated into distinct cell fates. The fact that these MADS domain proteins exhibit similar DNA-binding specificities *in vitro* suggests that their distinct *in vivo* biological activities may be conferred by other yet unknown genes. We set out to identify additional genes that play regulatory roles in the AG pathway with an enhancer screen using a weak ag allele, ag-4. We identified two genes, HUA1 and HUA2, as new members of the AG pathway. Genetic analyses suggest that HUA1 and HUA2 perform functions similar to those of AG in flower development. A genetic screen in the hua1-1 hua2-1 background resulted in the isolation of extragenic mutations (named the hen mutations) that enhance the hua1-1 hua2-1 mutant phenotypes. The hua1-1 hua2-1 hen mutants display novel floral homeotic phenotypes indicative a compromised AG pathway. The analysis of these mutants will allow us to start to dissect the floral homeotic AG pathway. The molecular nature of HUA1 and HUA2 and the preliminary analyses of the hua1-1 hua2-1 hen mutants will be presented.

339 The *Arabidopsis* STRUBBELIG gene encodes a distinct putative leucine-rich repeat receptor kinase involved in the control of cell morphogenesis

David Chevalier and Kay Schneitz*

Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland. *Email: Kay.Schneitz@access.unizh.ch

We are interested in the genetic and molecular basis underlying plant organogenesis. Here we present our analysis of the STRUBBELIG (SUB) gene. Plants defective for SUB function show several features: female sterility, reduced stature, altered shape of floral organs and twisted stems. With respect to ovules the development of the outer integument is altered. Some regions stop their development after they get initiated whereas others undergo uncoordinated growth resulting in characteristic outgrowths. Furthermore, compared to wild type the epidermis and cortex cells of sub inflorescence stems are irregular shaped and the intercellular spaces are reduced. We have cloned the SUB gene by chromosome walking. Northern blot analysis showed SUB to be ubiquitously expressed. *In situ* hybridization experiments revealed that SUB mRNA accumulates in the inflorescence and floral meristems. It is strongly expressed in the growing zone of immature floral organs and ovules. Later during ovule development SUB transcripts preferentially accumulate in the outer integument. Taken together, the sub mutant phenotype and the expression pattern implicate a role for SUB in cell morphogenesis by regulating either cell division and/or cell expansion. SUB encodes a putative

transmembrane leucine-rich repeat (LRR) receptor kinase of the serine/threonine kinase family. Three homologues in *Arabidopsis thaliana* and in *Zea mays* are known. All of them share the same overall organization at the protein level. They also show specific features as a small number of LRRs (five to seven), an proline rich region close to the membrane-spanning region and a new domain at the beginning of the extra cellular region. This domain was called SUB domain. Thus, SUB is a member of a small but distinct family of LRR receptor proteins and defines a novel signaling pathway regulating cell morphogenesis in *Arabidopsis*.

340 Genetic and molecular analysis of female gametophyte mutants in *Arabidopsis*
Cory A. Christensen, Ryan Brown, April Sullivan, Jessica Gold, and Gary N. Drews
Dept. of Biology, University of Utah, 257 So. 1400 E., Salt Lake City, UT 84112-0840
The female gametophyte is a seven-celled, eight-nucleate structure which plays a central role in angiosperm reproduction. Following fertilization, the egg cell and central cell develop into the embryo and endosperm, respectively, of the seed. In addition to giving rise to a seed, the female gametophyte attracts a pollen tube, mediates fertilization, and inhibits seed development in the absence of fertilization. To identify the molecules that mediate these female gametophyte functions and regulate female gametophyte development, we have conducted a genetic screen. Female gametophyte mutants are identified by reduced seed set and reduced transmission of mutant alleles through the female. Approximately 0.8% of T-DNA mutagenized lines exhibit both of these phenotypes. To determine which process is affected in these mutants, we examine developing, mature, and fertilized female gametophytes using confocal laser scanning microscopy and we determine the identity of the disrupted genes using TAIL-PCR. Using this data, we have identified molecules required for specific steps in female gametophyte development and function. Phenotypic analysis and gene identity will be presented for a collection of approximately 40 mutants. One of these, *gfa2*, affects the nuclear fusion step of female gametophyte development. In wildtype, the central cell inherits two nuclei, called the polar nuclei, which migrate together and fuse to form the secondary endosperm nucleus. In the *gfa2* mutant, the polar nuclei migrate and make contact, but fail to fuse. The *GFA2* gene, which rescues the *gfa2* mutant, encodes a protein with strong homology to DnaJ. DnaJ proteins have been linked to the process of nuclear fusion by studies of yeast karyogamy mutants. When either of two *S. cerevisiae* genes, *SEC63* or *JEM1*, which encode DnaJ-type proteins, are mutated the two nuclei of the mating cells make contact but fail to fuse. A detailed analysis of the *gfa2* mutant will be presented.

341 Progress Toward The Positional Cloning Of The *Nicotiana glauca* Pollen-S Gene
Jeff Coleman, Adrienne E. Clarke, and Ed Newbigin
Plant Cell Biology Research Centre, School of Botany, The University of Melbourne
Self-incompatibility in solanaceous plants is genetically controlled by a single S locus with many alleles. If the S allele carried by the haploid pollen grain matches either S allele expressed by the diploid style, the pollen tube is rejected. Pollen tubes expressing an S allele different to the S alleles expressed by the style are not rejected and grow through the style to the ovary. An S locus gene encoding an allelic series of extracellular ribonucleases called the S-RNases controls the self-incompatibility response of the style. The gene that controls the self-incompatibility response of pollen is unknown.

We have a population of *Nicotiana glauca* plants segregating for a mutation that only affects the self-incompatibility response of pollen. Each mutant plant contains an extra S allele and the mutation only arises in pollen that carries two different S alleles. We used the amplified fragment length polymorphism technique to identify markers near the extra S allele. Twenty-two AFLP fragments were identified, cloned, sequenced and used to probe blots of *N. glauca* DNA. Four fragments hybridized to low-copy sequences and the rest hybridized to repetitive DNA. The sequence of one of the four fragments had similarity to an EST in the database but northern analysis revealed this fragment was expressed in all tissues except pollen grains. These markers can be used to physically map the *N. glauca* S locus.

342 A cytological study throughout the time course of fertilization in *Arabidopsis thaliana*

Jean-Emmanuel Faure and Christian Dumas

UMR 5667, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69364 Lyon, Cedex 07, France

The cytology of the female gametophyte has been reported in detail in *Arabidopsis thaliana* (Christensen et al, 1997, *Sex. Plant Rep.* 10:49). The main cytological events during embryo and endosperm development have also been studied quite extensively (reviewed by Laux and Jürgens, 1997, *Plant Cell* 9:989; Berger, 1999, *Curr. Op. Plant Biol.* 2:28). However, there are fewer observations reported on fertilization *sensu stricto* and the very early events following gametic fusion. Here we have analyzed *Arabidopsis* fertilization using Confocal Laser Scanning Microscopy. Pistils were fixed at various times after controlled pollination in wild type plants (Colombia and C24 ecotypes) and observed using a simple protocol described by Christensen et al (1997, *Sex. Plant Rep.* 10:49). Serial optical sections were taken from about 500 ovules. We show that one of the two synergid cells degenerates 7 hours after pollination. We observed a change in the polarity of the egg following the degeneration of the synergid, which may constitute a very early post-fertilization event, before karyogamy. Karyogamy seems to occur 8 to 9 hours after pollination, and therefore less than two hours after gametic fusion. This was detected by the presence of two nucleoli of different diameters in both the egg nucleus and the central cell nucleus. The early steps of fertilization seem therefore to be similar in *Arabidopsis* and in other plants such as maize for which the time course has already been studied in detail (Mòl et al, 1994, *Plant J.* 5:197). The first nuclear division took place rapidly in the fertilized central cell, probably within the hour following karyogamy. Two nucleoli were then observed transiently in each nucleus produced by this first nuclear division, which occurs 9 to 12 hours post-pollination. A similar two nucleoli were also transiently observed following the second nuclear division which occurs more than 12 hours post-pollination. No significant differences for any of these observations were noted between different ecotypes (C24 and Colombia) or growth conditions (greenhouse or growth chamber). These observations constitute a basis for the further phenotypic analysis of mutants affected in fertilization *sensu lato*.

343 Cloning of CER6, a gene required for fertilization in *Arabidopsis*, and analysis of suppressors

Aretha Fiebig*, Jacob Mayfield#, Natasha Miley#, Samantha Chau#, Robert L. Fischer+ and Daphne Preuss#

*Dept. Biochemistry and Molecular Biology, #Dept. Molecular Genetics and Cell Biology, University of Chicago, 1103 E 57th Street, Chicago, IL +Dept. Plant Biology, University of California, Berkeley, 111 Koshland Hall, Berkeley, CA

The pollen coat of *Arabidopsis* contains critical components necessary for recognition by the female stigma cell and subsequent hydration of the pollen grain - events necessary for successful fertilization. The pollen coat is rich in lipids and proteins which are both important for the initial steps of fertilization. However, the function of the individual pollen coat components is not known. Mutations in the CER6 locus eliminate the majority of the pollen coat material including both lipids and proteins, and diminish the stem cuticular waxes. In particular, in *cer6* mutants lipids greater than 28 carbons in length are significantly reduced both in the pollen coat and the stem cuticle. Importantly, *cer6* plants are sterile under normal conditions, but the sterility is reversed under high humidity conditions where pollen can absorb ambient water thus bypassing the need for water from the stigma. To understand the nature of the genes which contribute to the pollen coat, we have positionally mapped and cloned CER6 - a very-long-chain fatty-acid condensing enzyme involved in the elongation of fatty acids. Additionally, we have identified the lesions in the *cer6-1* and *cer6-2* alleles. *cer6-1* is a 3 bp deletion resulting in the loss of residue E319 and *cer6-2* is a point mutation which changes H148 to a proline. Furthermore, we have identified an intragenic mutation which suppresses the sterility of the *cer6-2* mutation. The suppressing point mutation changes the *cer6-2* P148 to a serine which is presumably less detrimental than the original proline mutation. The *cer6* suppressed plants have partially restored pollen coats, allowing successful fertilization at low humidity. Interestingly, the stem cuticle is not restored in the suppressor. These results support the importance of lipids in the pollen coat and suggest generation of pollen coat and stem cuticle require different levels of CER6 activity.

344 Genetic Dissection of Gynoecial Medial Ridge Development

Robert G. Franks¹, Joshua Z. Levin², and Zhongchi Liu¹

1) Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742; 2) Novartis Agribusiness Biotech Research Inc, Research Triangle Park, NC

The medial ridge of the gynoecium gives rise to the placentae and associated ovules, and to the septal transmitting tract. These structures are critical for female reproductive competence. We demonstrate that three genetic regulators, LEUNIG(LUG), LEUNIG-LIKE (LGL), and AINTEGUMENTA (ANT), all play important roles in the development of the medial ridge and derived tissues.

Mutations in *lug* and *lgl* share many phenotypic similarities including a reduction in floral organ number, ectopic expression of AGAMOUS (AG), homeotic transformations of floral organ identity and partially unfused gynoecia. These defects are enhanced in *lgl lug* double mutants. These data suggest that LGL and LUG encode closely related and partially redundant genetic functions. LUG encodes a nuclear-localized WD-40 repeat-containing protein that shares sequence similarity with the yeast TUP1 transcriptional co-repressor. The LGL gene is not yet isolated, but likely encodes a novel gene that maps to chromosome 1, about 3 cM south of GAPB.

Another similarity between *lgl* and *lug* is the nature of their interaction with *ant*. Both the *ant lug* and *ant lgl* double mutants display a dramatic enhancement of gynoecial defects that includes a complete loss of the medial ridges, as well as the ovules, septa and stigma. This medial ridge phenotype is specific for *ant lug* and *ant lgl* double mutants. It is not seen in *lgl lug* double mutants, nor in double mutants of other negative regulators of AG. Thus we have defined two genetic activities required for medial ridge development: one activity encoded by the ANT gene and another encoded by LGL and LUG. Only when both of these activities are affected (as in *ant lug* or *ant lgl* double mutants) do we observe a complete absence of the medial ridge.

ANT encodes a transcriptional regulator that likely regulates cell proliferation within developing organ primordia. Its activity may be needed to maintain the meristematic nature of the developing medial ridge. The specific interaction of LGL and LUG with ANT in the medial ridge suggests that LGL and LUG may also play an important role in cell proliferation, either in close functional association with ANT or by regulating common target genes.

345 DGG (DEGENERATE GAMETOPHYTE), a novel gene with potential roles in male and female gametophyte development

Golan, T. and Fernandez, D.E.

Dept. of Botany, Univ. of Wisconsin-Madison 430 Lincoln Dr. Madison, WI 53706

Genes that are required for gametophyte (haploid) viability are difficult to recover by conventional mutant screening. Therefore, relatively few such genes have been characterized. We identified DGG (DEGENERATE GAMETOPHYTE), a novel gene that we believe to be involved in gametophyte development, in a PCR-based differential-display screen for embryo-expressed mRNAs in *Brassica napus*. The Arabidopsis ortholog of the Brassica gene DGG maps near API on the bottom arm of chromosome 1. In Brassica, DGG is preferentially expressed in floral apices, young floral buds and embryos. Arabidopsis plants transformed with a DGG-?-glucuronidase translational fusion construct show GUS staining in developing floral buds, developing embryos and roots of young seedlings. To study the function of DGG, Arabidopsis plants were transformed with a vector designed to express Brassica DGG cDNA sequence along with a 150bp inverted repeat. Similar constructs have been shown to cause sequence-specific post-transcriptional gene silencing in other systems. We recovered six independent lines that showed a significant reduction in seed set. Closer inspection revealed that up to 90% of both the male and female gametophytes were defective. Initial observations indicate that spores are produced in both anthers and ovules, but the gametophytes fail to develop normally. At floral maturation (stage 13), the majority of the pollen consists of collapsed, empty shells. Ovules contain a highly vacuolated, strongly staining body instead of a normal female gametophyte. Based on the predicted amino-acid sequence, DGG encodes a soluble protein of 25KDa with a putative myristoylation site. DGG's sequence is largely novel but shares similarity to *C. elegans*, yeast, *Drosophila* and bacterial proteins in at least one region. The function of this region and of these proteins is unknown. Based on our studies, we suggest that DGG function may be important for the development and/or viability of plant gametophytes. Funded by the UW-Madison Graduate School, U.S. Dept. of Agriculture (96-35304-3699) and by DOE/NSF/USDA Collaborative Program on Research in Plant Biology (DBI 96-02222).

346 Polycomb-group gene function in Arabidopsis

Justin Goodrich, C. MacDougall, L. Primavesi, C. Stock, Y. Chanvivattana, S. Mora-Garcia, C. Wilson

University of Edinburgh, Institute of Cell and Molecular Biology

Polycomb-group (Pc-G) genes act as transcriptional repressors in plants and animals and regulate homeotic and other developmentally important target genes. In Arabidopsis, the CURLY LEAF (CLF) gene encodes a homologue of the Drosophila Pc-G member Enhancer of zeste, and is required for transcriptional repression of the floral homeotic genes AGAMOUS (AG) and APETALA3 (AP3). To understand better the action of CLF, we are characterising its protein localisation and its interactions with other Pc-G members.

The CLF gene is transcribed ubiquitously in flowers and inflorescences, yet its action as a repressor of AG is spatially localised. To characterise CLF protein distribution, we have raised polyclonal antibodies to CLF, and constructed transgenes that express fusions of CLF to green fluorescent protein (GFP). Expression of GFP-CLF fusions in transgenic plants is sufficient to complement null *clf* mutations, indicating that the fusion retains CLF+ function. We will present the localisation of CLF in transgenic Arabidopsis and tobacco cell lines.

Unlike in animals, where Pc-G mutants are embryo lethal, even null *clf* alleles confer relatively mild phenotypes. To test whether CLF shows functional redundancy with other plant Pc-g members, we have been conducting genetic and molecular screens for modifiers. We have characterised a CLF homologue, CURLY-LEAF LIKE1, and will present single and double mutant phenotypes that suggest that these two genes show functional redundancy, and therefore that CLF has a broader role than was evident from single mutant phenotypes alone.

347 Genetic and molecular analysis of gametophyte development and function

Paul E. Grini, Gerd Jurgens and Martin Hulskamp

ZMBP, University of Tuebingen, Germany

The life cycle of plants comprises two alternating generations, the diploid sporophyte and the haploid gametophyte. A genetic analysis of gametophyte development and function has partly been hampered by the difficulty of identifying haplo-phase-specific mutants in conventional mutagenesis screens.

We employ two genetic strategies to screen for mutants affecting the gametophytic phase in Arabidopsis: (1) The first approach is based on segregation distortion of visible markers if an EMS-induced haploid lethal mutation has been induced in the proximity of one of the markers or its wild-type homologue. Using a multiple marker chromosome we have isolated more than 100 mutant lines that display altered segregation of markers. A genetic analysis of these lines is in progress and shows a marked reduction of the transmission of the male and/or female gametes. At a saturated level this strategy allows an estimation of the number of male, female or both male and female genes expressed in the gametophytic phase. (2) The second approach is using lines from an AcDs Transposon mutagenesis, and is based on segregation distortion of Kanamycin resistance if the insertion is in a gene required for the gametophytic phase. Several mutants showing

blocked transmission of the Kanamycin resistance through the male or the female gametophyte have been isolated and molecular characterisation is in progress. Three classes of mutants can be expected to be found in such linkage based screens: Mutants affected in gametophyte development; in the fertilization process or in zygotic development. Here, we will report on mutants representative for all three classes.

Grini et al Genetics 151, 1999

348 Control of Arabidopsis Embryo Development

John J. Harada

Section of Plant Biology, Division of Biological Sciences, University of California, Davis, CA 95616

Seed development is initiated with a double fertilization event, resulting in the formation of the triploid endosperm and the diploid zygote. The zygote then enters a phase of morphogenesis in which the body plan of the plant is established, tissue systems are differentiated, and embryonic organs are formed. Late in embryogenesis, embryos undergo the maturation phase during which storage macromolecules accumulate and the ability to withstand desiccation is acquired. Little is known at a mechanistic level about the processes that initiate embryo formation and coordinate early and late embryogenesis. We are studying the Arabidopsis LEAFY COTYLEDON (LEC) genes that play a central role in integrating several distinct aspects of embryogenesis. The LEC genes, LEC1, LEC2, and FUSCA3, are required to maintain suspensor cell fate, to specify cotyledon identity, to initiate and/or maintain the maturation phase, and to suppress postgerminative development during embryogenesis. The LEC genes are unique among other known embryonic regulators in that they function during both the early morphogenesis and late maturation phases of embryogenesis. We have shown that LEC1 is homologous to a conserved eukaryotic transcription factor. Our studies suggest that LEC1 plays a critical role in embryogenesis by establishing an environment that allows embryo development to occur. I will discuss our efforts to dissect LEC1 function and to identify genes encoding proteins with similar functions.

349 TSO1 is essential for ovule and floral morphogenesis

Bernard A. Hauser,^{1,2} Sung O. Park¹ and Charles S. Gasser²

¹Department of Botany, University of Florida, Gainesville, FL 32611; ²Section of Molecular and Cellular Biology, University of California, Davis, CA 95616

Morphogenesis is dependent on appropriate directional cell expansion and cell division. These processes are precisely regulated during ovule morphogenesis so tissues and organs form at proper locations and in the appropriate orientation. The variety of mutant defects that control morphogenesis indicates that this process is largely under genetic control. Analyses of *tso1* mutants reveal a loss of control of directional cellular expansion and coordination of growth of adjacent cells, and defects in karyokinesis and cytokinesis have been reported. We isolated TSO1 by a map-based approach, and show that it is a member of a family of at least three genes in Arabidopsis. The putative TSO1 protein contains a nuclear localization signal and TSO1-fusion proteins are found in the nucleus. The putative TSO1 protein has two cysteine-rich regions that are similar to the CXC domains of a variety of proteins from plants and animals, including a class of kinesins

involved in chromosome segregation, and enhancer of zeste-type proteins. The *tso1-3* allele exhibits defects in ovule development, including a failure in megasporogenesis. These ovule defects result from a premature stop codon partway through the *tso1-3* transcript. The *tso1-1* allele contains an amino acid substitution in the well-conserved CXC domain, indicating this domain is crucial for TSO1 function. The amino acid substitution in *tso1-1* plants leads to a stronger phenotype than is found in *tso1-3* plants. Flower development in *tso1-1* plants is aberrant, producing flowers with rudimentary and malformed petals, stamens, pistils and ovules. Consistent with these phenotypes, TSO1 transcript was most abundant in flowers, where it accumulated to the highest levels in developing ovules and microspores. The *tso1* mutant phenotypes and the novelty of the TSO1 sequence suggest the existence of previously unknown participants in the morphogenesis of eukaryotic cells.

350 Analysis of gene expression patterns in the shoot apical meristem

Martin Hobe, Ulrike Brand and Rüdiger Simon

Institut für Entwicklungsbiologie, Universität zu Köln, Gyrhofstr.17, 50923 Köln, Germany

The expression patterns of most of the known genes involved in meristem establishment and maintenance are laid down in embryogenesis and remain widely unchanged through the entire life cycle of the plant. Cells being displaced by directional cell divisions in the shoot apical meristem are passing those expression domains and thereby change their gene expression profiles, possibly in order to acquire the ability to adopt different cell fates. To address the question which factors are responsible for the establishment and stability of those domains, we used a UFO-promotor-GFP-fusion and have begun to analyse the changes of UFO-GFP-expression in diverse meristem mutants. One of the crucial aspects of meristem maintenance is to keep the balance between the amount of pluripotent stem cells and differentiated cells. In the Arabidopsis shoot apical meristem, the CLAVATA genes CLV1, CLV2 and CLV3 negatively regulate the size of the stem cell harbouring central zone, whereas WUSCHEL (WUS) seems to be a positive regulator of stem cell fate in cells overlying its expression domain. We have analysed in detail the CLV3 expression pattern in the wild type and a collection of meristem mutants by RNA-in situ hybridisation and a CLV3-promotor-GUS-fusion. Further-more, we have analysed the expression patterns of other genes that possibly play a role in patterning the shoot apical meristem in the wildtype and diverse mutant backgrounds.

351 The Arabidopsis MCM gene PROLIFERA is expressed in dividing cells and is maternally required for early development

David R. Holding, Robert A. Martienssen and Patricia S. Springer

Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124, USA

The PROLIFERA (PRL) gene of Arabidopsis thaliana encodes a member of the conserved MCM protein family that functions in DNA replication. PRL is expressed in dividing cells during the vegetative and reproductive phases of the Arabidopsis life cycle. During female reproductive development, PRL is strongly expressed in developing carpels and ovule primordia. In mature ovules, expression persists in the central cell nuclei, synergids and inner integument. After fertilization, PRL is strongly expressed in

endosperm nuclei both prior to and immediately after cellularization. PRL is also expressed strongly in developing pollen despite the fact that pollen development is unaffected by the prl mutation. PRL activity is required for proper megagametophyte, embryo and endosperm development and PRL is maternally required such that crosses between heterozygous prl/+ females and wild type males may result in embryo abortion . Despite this maternal effect, both maternal and paternal PRL alleles are equivalently expressed in both embryo and endosperm from the early stages of development (Springer et al., 2000. Development 127, 1815-1822). Aborted embryos show a range of phenotypes, including aberrant cell division orientations, which will be described.

352 AGAMOUS Regulation by LEAFY

Ray L. Hong^{1,2}, Maximilian A. Busch¹, and Detlef Weigel¹

¹Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037; rhong@biomail.ucsd.edu; <http://www.salk.edu/LABS/pbio-w/>; and ²Department of Biology, UC San Diego, La Jolla, CA 92093

The LEAFY (LFY) transcription factor is a direct regulator of the floral homeotic gene AGAMOUS (AG) (Busch et al., Science 285:585 [1999]). However, it is not known how LFY, which is expressed throughout the floral primordia, specifically activates AG only in a subset of cells in the inner whorls. Using a reporter-based screen, we have set out to find and characterize LFY cofactors involved in positively regulating AG. The mutagenesis used an 800 bp early active, LFY-dependent AG enhancer element driving a GUS reporter. We found one mutant with reduced early GUS activity. This mutant also shows a floral phenotype, and has lateral stamens transformed into petals. Two other mutations increase AG::GUS expression, but do not cause changes in floral morphology. In addition to the forward genetic approach, we are analyzing possible important regulatory sites in the AG enhancer close to the LFY binding sites. These include a CARG-box, which can be bound by MADS domain proteins, as well as a highly conserved region that includes two CCAAT-boxes, which are bound by heterotrimeric HAP complexes. Preliminary results suggest that the CCAAT boxes are required for increasing the levels of AG enhancer activity, although they are not essential for enhancer activity. Surprisingly, mutating the CARG box results in ectopic AG enhancer activity in the shoot apex and very young floral primordia. We have also further defined the biochemical properties of LFY, including identification of the DNA-binding domain, and demonstrating dimerization in a newly developed yeast-reporter system.

This work has been supported by an NIH training grant (R.H.), an HFSP Long-Term fellowship (M.A.B.), and a grant from the DOE Division of Energy Biosciences (D.W.).

353 Overexpression of a cytochrome P450 gene, CYP78A9, induces large and seedless fruits in Arabidopsis

Toshiro Ito and Elliot M. Meyerowitz

California Institute of Technology

An activation tagging screen in which the Cauliflower Mosaic Virus 35S enhancer was inserted randomly into an Arabidopsis thaliana genome homozygous for the floral homeotic mutation apetala2-1 (ap2-1) resulted in a line (28-5) with extraordinarily wide, heart-shaped ovaries. The ovary of the 28-5 ap2-1 mutant shows an oval shape due to

increased numbers of enlarged cells. When the *ap2-1* mutation is crossed out of the genetic background, more elongated rather than wider fruits are obtained. Normally, *Arabidopsis* fruits will only develop to a normal size when the ovules are present and fertilized. In the 28-5 single mutant, the siliques keep growing despite failure of fertilization and can reach nearly normal size. When wild-type pollen was used to pollinate the mutant pistil, the pollinated 28-5 silique became more than 10% longer and 40% wider than a wild-type silique, although producing very few seeds. The enhancer insertion in line 28-5 acts by hyperactivating a cytochrome P450 gene, *CYP78A9*. The pistil of 28-5 *ap2-1* mutant flowers shows a structure similar to that of *Capsella bursa-pastoris*, a distant mustard relative of *Arabidopsis thaliana*, suggesting that the processes regulated by the *CYP78A9* protein may be involved in evolutionary control of carpel shape.

354 *Arabidopsis* mutants defective in the interactions between pollen tube and female tissues

Masahiro Kanaoka, Kentaro K. Shimizu and Kiyotaka Okada

Department of Botany, Graduate School of Science, Kyoto University

In sexually reproductive plants, successive processes of mutual signal exchange between male and female are important events for fertilization. However, little is known about the molecular mechanisms. To investigate these mechanisms, we examined growth of pollen tubes in self-pollinated pistils of M1 and M2 plants (total 10,000 pistils) mutagenized by EMS using fluorescence microscopy after staining with aniline blue. We obtained mutants with defects in the following steps. 1) Pollen adhesion to the stigma; in the case of the *kompeito* mutant, the pollen shape is irregular. 2) Growth of pollen tubes; pollen tubes of *nobinai* mutant stop their growth in the middle part of the pistil. 3) Penetration of a pollen tube into an ovule; the integument of *int2* mutant ovule is short and the female gametophytes are often naked. Pollen tubes sometimes fail to penetrate into the exposed female gametophytes. Similar phenotype was observed in the *in vitro* pollen tube guidance for *Torenia* naked ovules. We propose that the role of integuments as a scaffold for pollen tube penetration. 4) Recognition of the female gametophyte by the pollen tube; the pollen tubes of the *karakusa* mutant continue their growth after arrival to ovules. The phenotypes of the *nobinai* mutant and of the *karakusa* mutant are observed when *Arabidopsis* pistil was crossed with pollens of closely-related species. This suggests that the divergence of the *NOBINAI* and *KARAKUSA* genes might have key roles in the process of divergence of Brassicaceae plants. (see abstract by K.K.S. and K.O.) We also have isolated several integument mutants. Some mutants had short outer integument, others had short inner integument. Half of the ovules in a pistil of the *int1* mutant have short outer integument. When mutant pollen was crossed to the wild type plants, an F1 plant showed the mutant phenotype. This indicates that *int1* is a gametophytic mutant. It is suggested that the female gametophyte emits some signal that stimulates the growth of the surrounding parental tissues.

355 Genomic imprinting of the *MEDEA* polycomb gene in the *Arabidopsis* endosperm

Tetsu Kinoshita, Ramin Yadegaria, Oliver Laoa, John J. Haradab, Robert B. Goldbergc, Robert L. Fischera

aDept. Plant & Microbial Biol., Univ. California Berkeley., bSect. Plant Biol., Div. Biol. Sci., Univ. California Davis., cDept. Mol. Cell & Dev. Biol., U.C.L.A

Genomic imprinting is when maternal and paternal alleles are marked and expressed differently. Imprinted gene expression has been discovered in mammals in the extra embryonic tissues that transfer nutrients from the mother to the developing embryo. Indirect genetic evidence suggested that imprinted genes also play a role during plant reproduction, as well. Here we report that the Arabidopsis MEDEA (MEA) gene is imprinted specifically in the endosperm, a tissue that may be functionally analogous to the mammalian extra embryonic membranes. MEA encodes an Arabidopsis SET domain polycomb protein. Inheritance of a maternal loss-of-function *mea-3* or *mea-4* allele results in embryo abortion and prolonged endosperm production, even when the paternal MEA allele is wild-type [PNAS (1999) 96:4186-4191]. To understand the molecular mechanism responsible for these parent-of-origin effects of *mea* mutations on seed development, we compared the expression of maternal and paternal MEA alleles. Only the maternal MEA mRNA was detected in the endosperm from seeds 6 days after pollination (torpedo stage) to 8 days after pollination (early maturation stage). By contrast, expression of both maternal and paternal MEA alleles was observed in embryos 6, 7 and 8 days after pollination, and in seedling, leaf, stem, and root. Thus, MEA is an imprinted gene where the paternal allele is silenced specifically in the endosperm. Gene silencing is often associated with an increase in DNA methylation. To understand the mechanisms of MEA gene silencing, and to identify where and when silencing is established and maintained, we examined the effects of mutations in the DDM1 (decrease in DNA methylation) gene on the *mea* mutant phenotype. The DDM1 gene encodes a protein with homology to the SNF2/SWI2 chromatin remodeling factor and reduces genome methylation. Analysis of progeny from reciprocal crosses between *mea-3*/MEA-3 and *ddm1/ddm1* parents suggests that DDM1 activity is required for the establishment of paternal MEA silencing during pollen development.

356 Pollen cell wall patterning in Arabidopsis by DEX1

Elizabeth Kostic, Dawn Paxson-Sowers, Chris Makaroff

Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056

Pollen cell walls consist of two layers. The outer layer, or exine, is made up primarily of sporopollenin and is extensively sculpted in a species-specific manner. The inner layer, or intine, is composed of proteins. While pollen wall formation has been described in detail, the mechanisms underlying exine patterning are not well understood. In the early stages of pollen cell wall development in Arabidopsis, the primexine matrix, the exine precursor, is deposited between the plasma membrane and the callose wall of the tetrad. The plasma membrane then invaginates producing a regular landscape of hills and valleys. Tapetally derived sporopollenin localizes to the peaks of these hills to begin exine patterning. Male sterile mutants of Arabidopsis that exhibit defects in pollen wall formation are being characterized in order to identify proteins responsible for this process. One line currently under characterization is *dex1*. Plants containing the *dex1* mutation are defective in exine patterning due to the lack of sporopollenin localization and attachment to the plasma membrane. Analysis of DEX1 suggests that it is a calcium binding protein that may localize to the plasma membrane. Results providing insight into the role of DEX1 in pollen wall and exine pattern formation will be presented.

357 Regulation of the MADS domain factor AGL15: one gene, two proteins?

Melissa D. Lehti-Shiu, and Donna E. Fernandez

Department of Botany, University of Wisconsin, Madison, WI 53706

AGL15 (AGAMOUS-like 15), one of the more divergent members of the MADS domain family of regulatory factors, accumulates in the nuclei of all embryo cells in Brassica and Arabidopsis. Immunolocalization experiments showed that the nuclear localization of AGL15 is developmentally regulated. Before fertilization, AGL15 is present in the cytoplasm. It becomes nuclear localized early in embryo development [Perry et al. 1996. *Plant Cell* 8: 1977-1989]. From immunoblot analysis, we have obtained evidence that there are two forms of AGL15 with different apparent molecular weights (approximately 26 kD and 38 kD in Arabidopsis). The 26 kD form is associated with soluble extracts, and the 38 kD form with nuclear extracts. AGL15 is a single copy gene in Arabidopsis, raising the possibility that AGL15 is post-transcriptionally or post-translationally modified. We are currently studying the accumulation of AGL15-T7 tag fusion proteins in transgenic Arabidopsis to establish the relationship between the two forms. Future plans include defining the regions of AGL15 that are necessary for cytoplasmic vs. nuclear localization. To obtain an *agl15* null background for these studies, and to define AGL15's role in plant development, we have screened for T-DNA insertions in the AGL15 locus. One insertion has been identified 200 bp upstream of the translational start site, and another has been identified in the first intron. We are currently examining the effects of these insertions on AGL15 protein accumulation and plant development. Funded by the UW-Madison Graduate School, U.S. Dept. of Agriculture (96-35304-3699) and by DOE/NSF/USDA Collaborative Program on Research in Plant Biology (DBI 96-02222).

358 A bHLH gene, INDEHISCENT1, is required for fruit dehiscence and mediates the fruitfull phenotype

Sarah Liljegen, Sherry Kempin, Alice Chen, Adrienne Roeder, Sonia Guimil, Tuky Khammungkhune, and Marty Yanofsky

Section of Cell and Developmental Biology, University of California at San Diego, La Jolla, California 92093-0116, USA

Fruit dehiscence occurs late in the development of dry, dehiscent fruit to ensure seed dispersal. We have been investigating regulation of this process through studies of shatterproof (*shp1 shp2*) fruit, molecular analyses of valve margin markers and genetic screens for additional indehiscent mutants. Because expression of the GT140 marker is largely absent from the valve margins of *shp1 shp2* fruit, we expected that the gene corresponding to this marker might also be involved in valve margin development and be required for fruit dehiscence. We have now determined that GT140 corresponds to a predicted bHLH transcription factor, named INDEHISCENT1 (IND1), as four mutant alleles of IND1 were also identified through our genetic screens. Like *shp1 shp2* fruit, *ind1* fruit do not show dehiscence zone differentiation, and in addition, valve margin lignification and expression of certain valve margin markers are more severely altered in *ind1* fruit compared to *shp1 shp2* fruit. We have also discovered that mutations in IND1 dramatically suppress aspects of the fruitfull (*ful*) mutant phenotype, that many T1 plants constitutively expressing IND1 produce fruit similar to *ful* fruit, and that some

35S::IND1 plants show unexpected phenotypes which suggest that IND1 may have other roles besides regulating valve margin development and fruit dehiscence.

359 Negative Regulation of AGAMOUS by LEUNIG, LEUNIG-LIKE, and LARSON During Flower Development

Zhongchi Liu¹, Joann A. Conner^{1,2}, Robert G. Franks¹, and Xiaozhong Bao¹

¹. Dept. of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742 ². Dept. of Horticulture, Univ. of Georgia, Tifton, GA 31793

Flower development in *Arabidopsis* is controlled by the activity of homeotic genes that specify floral organ identity and by the cadastral genes that regulate the spatial and temporal expression domains of the homeotic genes. LEUNIG (LUG) and LEUNIG-LIKE (LGL) are both cadastral genes involved in establishing the boundary of AGAMOUS (AG) expression during flower development. *lug* mutations result in ectopic and precocious expression of AG in a developing flower and the conversion of sepals to carpels and petals to stamen-like filaments or absent. *lgl* mutants exhibit a similar but less severe phenotype to *lug*. *larson* (*lsn*) mutation enhances both *lug* and *lgl* but exhibits no phenotype on its own. Thus, all three genes are involved in the negative regulation of AG with LUG playing a leading role. Using a map-based approach, we isolated the LUG gene and showed that the LUG protein has a glutamine-rich region at the N-terminus and six WD repeats at the C-terminus. The presence of these motifs and their relative position within a gene is similar to the well-studied transcriptional repressors Groucho in *Drosophila* and TUP1 in yeast. These co-repressor proteins are known to bind to specific DNA-binding factors to effect downstream gene expression. LUG, thus, may exert its function via a similar mechanism. Consistent with its role as a regulatory protein, the LUG-GFP fusion protein is localized in the nucleus. However, LUG mRNA is expressed in all four whorls of a flower, suggesting that the region-specific activity of LUG may be dependent on additional regional-specific factors. Additional molecular analyses of LUG and the molecular cloning of LGL and LSN are in progress. By focusing on understanding how LUG, LGL and LSN regulate AG, we hope to illuminate the mechanism underlying region-specific gene expression for flower pattern formation.

360 Interactions, pattern of expression, and imprinting of FIS2, MEDEA and FIE

Ming Luo, Pierre Bilodeau, E. Dennis, J. Peacock and A. Chaudhury

CSIRO Plant Industry, GPO BOX 1600, Canberra, ACT 2601, Australia

We studied the expression of MEA, FIS2 and FIE genes during reproductive development using the reporter gene -glucuronidase (GUS) fusion. FIS2 is expressed in each of the nuclei of the central cells before fusion and after pollination maternally derived FIS2 gene expression persists in the free nuclear endosperm and is not expressed in cellularised endosperm. MEA expression is similar to but more diffuse than FIS2 expression. FIE expression is also observed in the female gametophyte before pollination and is present in embryo and endosperm after pollination. Other sporophytic tissues also show FIE expression. FIS2, MEA, and FIE show parent of origin effects of gene expression in at least some stage of endosperm development. Only maternally derived FIS2, MEA, and FIE show expression in early endosperm. Paternally derived FIS2, MEA, and FIE expression is absent in early endosperm, indicating that FIS2, MEA, and FIE are imprinted. Female gametophytic embryo arrest caused by *fis2*, *mea* and *fie* is

rescued by pollen from a plant expressing an antisense to DNA methyltransferase. The wild type ovules give small seeds if pollinated with demethylated pollen. In a yeast two hybrid system MEA interacts with itself and with FIE. FIS2 does not interact with either MEA or FIE. The similarity of phenotypes of these three mutants and the fact that the FIE, MEA and FIS2 genes are expressed in the same cells support our model describing a FIS complex as a repressor of key seed development genes.

361 *Arabidopsis thaliana* pollen coat proteins are essential for efficient pollination
Jacob A. Mayfield and Daphne Preuss

The University of Chicago, 1103 E. 57th Street, EBC 304, Chicago, IL 60637

The molecules necessary to initiate pollination in a dry environment are contained in the lipid- and protein-rich matrix surrounding *Arabidopsis thaliana* pollen. This pollen coat performs at least two functions: regulating the onset of hydration and facilitating the rapid transfer of water. Despite the fundamental importance of this stage in plant development, mutations that alter individual pollen coat components have proved difficult to find. Coupling biochemical purification with protein microsequence, we identified all major pollen coat proteins and have isolated insertion mutations in several to address their function. Previous reports focused on the role of pollen coat lipids; our data demonstrate pollen coat proteins play an important role in the hydration process. The pollen coat protein GRP17 contains an oleosin domain, intriguing because oleosin proteins in the seeds of oil seed plants have been shown to regulate lipid behavior. A mutation in the GRP17 gene delayed the hydration of pollen grains contacting the stigma, decreasing the ability of mutant pollen to compete with wild-type. All previous hydration mutants affect wax biosynthesis, disrupting the entire pollen coat. *grp17-1* is unique, eliminating a single determinant, uncovering a key regulatory element and illustrating the importance of proteins in hydration. To further dissect hydration phenomena, we have generated mutations in other pollen coat proteins and identified mutations that result in varied levels of lipids in the pollen coat. By addressing all pollen coat components, we hope to delineate how individual roles are combined to perform a complex process.

362 The TITAN5 Gene Encodes a Protein Related to the ADP Ribosylation Factor (ARF) Family of GTP Binding Proteins

John McElver¹, David Patton¹, Michael Rumbaugh², Chun-ming Liu^{2,3}, Li Jun Yang², Jia Qian Wu², Iris Tzafir², and David Meinke²

¹Novartis Agribusiness Biotechnology Research, Inc., R.T.P., NC; ²Department of Botany, Oklahoma State University, Stillwater, OK; ³Plant Research International, Wageningen Univ. Research, Wageningen, The Netherlands

The titan (*ttn*) mutants of *Arabidopsis* exhibit dramatic alterations in mitosis and cell cycle control during seed development. Endosperm development in these mutants is characterized by the formation of giant polyploid nuclei with enlarged nucleoli. Embryo development is also disrupted in most titan mutants and is accompanied by significant cell enlargement in *ttn1* and *ttn5*. We present here an overview of the 13 titan mutants and 9 complementation groups identified to date from collections of T-DNA insertion lines. Five of these genes are represented by tagged mutant alleles. We also describe in detail the molecular cloning of *TTN5* using a T-DNA tagged allele. A second *ttn5* allele with a similar phenotype contains a nonsense mutation in the same coding region. The

predicted protein is related in sequence to ADP ribosylation factors (ARFs), members of the RAS family of small GTP binding proteins that regulate a variety of cellular functions in eukaryotes. TTN5 is most closely related in sequence to the ARL2 class of ARF-like proteins isolated from humans, rats, and mice. Although the cellular functions of ARL proteins remain unclear, the *ttn5* phenotype is consistent with the known roles of ARFs in the regulation of intracellular vesicle transport.

363 Roles of INNER NO OUTER and SUPERMAN in ovule gene expression

1Meister, R. J., 2Bowman, J. L., and 1Gasser, C.S.

1Section of Molecular and Cellular Biology and 2Section of Plant Biology, University of California, Davis

Asymmetrical growth and development are essential in all higher organisms, and the molecular mechanisms governing these processes are beginning to be deciphered. INNER NO OUTER (INO), a member of the recently described YABBY gene family, is asymmetrically expressed in ovules and is believed to be one mediator of polar development in Arabidopsis. To elucidate the mechanism of its transcriptional regulation, a 2.0 kb fragment of genomic DNA from upstream of the INO translational start codon (P-INO) was isolated. Using P-INO to express the INO cDNA or β -glucuronidase (GUS) resulted in complementation of the *ino* phenotype and GUS expression identical to endogenous INO transcripts, respectively. Expression of the SUPERMAN (SUP) cDNA by P-INO, resulted in plants with *ino* ovules, confirming a previous model that SUP is a negative regulator of INO expression. CRABS CLAW (CRC), another YABBY family member, when expressed under the control of P-INO resulted in transgenic plants which phenocopy *sup* ovules. Since it has been hypothesized that the *sup* phenotype is due to expanded chalazal expression of INO, these results provide evidence that INO is involved in positive autoregulation that is interrupted by SUP. To determine which domain of INO is required to maintain its correct expression pattern, protein domain exchanges between INO and CRC have been performed. The resulting chimeras were expressed under the control of P-INO and their ability to complement the *ino* phenotype was assessed. The results indicate that some domains may be interchangeable between INO and CRC while others have gene-specific functions. In summary, we are formulating a transcriptional regulatory model of an asymmetrically expressed gene that involves autoregulation with attenuation by a known cadastral gene.

364 Morphological and Genetic Analyses of a T-DNA tagged Reduced Fertile Mutant of Arabidopsis Exhibiting Aberrant Exine Patterning

H.A. Owen¹, D.M. Paxson-Sowders² and C.A. Makaroff²

Department of Biological Sciences, University of Wisconsin - Milwaukee¹, Milwaukee, WI 53211 and Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056²

During morphological characterization of fertility-impaired mutant lines produced by T-DNA mutagenesis a line, designated 6491, was identified that produces pollen with aberrant exine. A comparison of wild type (WT) and mutant grains using scanning electron microscopy revealed that the mutant grains are more varied in size than WT pollen, rounded instead of elongate in shape, lack colpi and have surface morphology that appears as randomly oriented ridges instead of the regular geometric array of tectum

ridges characteristic of normal Arabidopsis final exine patterning. These grains appear able to effect fertilization, although with a lower rate of success than that achieved by WT pollen. The observed phenotype is reduced fertility rather than complete sterility and we are able to maintain this line as a homozygous recessive. The line contains a single complex T-DNA insert that is tightly linked to the mutation. Light microscopic observation of semi-thin (0.5 μ m) sections of buds show that the first difference between 6491 and WT pollen development occurs prior to meiosis when the callose wall secreted by 6491 microsporocytes is thinner than that observed in WT. Meiosis does not proceed normally. The resultant coenocytic microsporocytes contain varying numbers and sizes of "nuclei." Centripetal ingrowths of callose cleave the mutant microsporocytes and, as observed in previously studied meiotic mutants, the number and sizes of "microspores" produced from individual microsporocytes is variable.

365 POP2, a gene required for guidance of Arabidopsis pollen tubes, is similar to an enzyme required for biotin synthesis

Ravishankar Palanivelu, Laura K. Wilhelmi and Daphne Preuss

Department of Molecular Genetics and Cellular Biology, University of Chicago, Chicago, IL 60637

Successful fertilization in plants requires precise guidance of pollen tubes to the ovules; an event mediated by strong adhesion interactions and specific signaling events. Mutations in the pollen-pistil interaction gene, POP2, cause a specific and dramatic disruption of guidance: pollen tubes do not adhere to ovule tissue and fail to target the eggs. Crosses between mutant and normal plants show that this mutant is self-sterile: sterility occurs only when mutant pistils are pollinated by mutant pollen. To better understand the specific role of POP2 in pollen tube guidance, we cloned the POP2 gene by chromosome walking. A genomic fragment containing the cloned gene complements the pop2 allele, restoring fertility to mutant plants, providing additional evidence that the cloned gene was indeed POP2. RT-PCR also show that POP2 mRNA levels are significantly reduced in the mutant flower tissues compared to wildtype. Sequence analysis of the cloned gene shows that POP2 gene has strong homology to prokaryotic DAPA aminotransferases, an enzyme that catalyzes one of the final steps in biotin biosynthesis. These results raise the possibility that POP2 functions in biotin synthesis, and that biotin may play a role in pollen tube growth or in pollen tube guidance. A derived CAPS (dCAPS) marker that unambiguously identifies the POP2 wildtype and mutant alleles was used to explore the interaction between the pop2 allele and another POP gene, POP3. Results from these studies revealed that in addition to the guidance defect, pop2 mutant strains exhibit embryo lethality. The presence of pop3 suppresses the lethal phenotype, but not the guidance defect. This conclusion is supported by the observations that (i) homozygous pop3, but not heterozygous or wildtype pop3, was required for mendelian segregation of pop2 allele and (ii) a mutant copy of pop3 is required to recover pop2 homozygotes.

366 Identification and Characterization of AGL11 mutant alleles

Anusak Pinyopich¹, Gary Ditta¹, Elvira Baumann², Ellen Wisman², Marty Yanofsky¹

1Section of Cell and Developmental Biology, University of California at San Diego La Jolla, CA 92093-0116 USA; 2Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné Weg 10, D-50829 Köln Germany

Despite extensive screening, no single mutants have been identified which affect *Arabidopsis* ovule identity specifically (Development, 124:1367, 1997). This may be due to genetic redundancy, as in *Petunia hybrida* two closely related MADS-box genes, FBP7 and FBP11, specify ovule identity redundantly. FBP7 and FBP11 are closely related to AGL11, SHATTERPROOF1 (SHP1), SHATTERPROOF2 (SHP2) and AGAMOUS. All four genes are expressed in developing ovules, while AGL11 expression is specific only to ovules. Ovule defects are not seen in shp1 and shp2 single or shp1 shp2 double mutants. Using a reverse genetic approach, we have isolated a new agl11-1 allele which has the maize autonomous transposable element, En-1, inserted into the third exon. Subsequently we have isolated two additional stable alleles, agl11-2 and agl11-3, which have RNA splicing defects. All single agl11 mutants have no obvious ovule phenotypes. We are investigating whether the shp1 shp2 agl11 triple mutant will cause loss of ovule identity.

367 ELUCIDATING GENE EXPRESSION PATTERN AND FUNCTION USING ENHANCER AND GENE TRAPS

Sarojam Rajani, Megan Griffith and Venkatesan Sundaresan

Institute of Molecular Agrobiolgy. 1,Research link, National University of Singapore

We have used the Maize Ac/Ds family of transposon as enhancer and gene traps to generate transposon insertion lines in *Arabidopsis* (Sundaresan et al., 1995). By screening several independent insertion lines, we have identified lines showing diverse patterns of GUS expression, ranging from reproductive organs like egg apparatus, embryo sac to vegetative tissues like lateral roots, trichomes. Some of these expression patterns are also developmentally regulated. Such transposon insertion lines, with specific reporter gene expression pattern, can be used as valuable cell and tissue specific markers for developmental studies in *Arabidopsis*. Some of these insertion lines with restricted GUS expression will be presented. A Screen for new mutants, revealed an insertion line, SGT 10017, which showed an aberrant carpel phenotype. In *Arabidopsis*, the wild type gynoecium consists of two fused carpels. In SGT 10017, the gynoecium consists of four fused carpels instead of two and also ectopic carpels are present interior to the normal fourth whorl. The organ number and the structure of the organs in the first three floral whorls appear to be normal. The insertion line showed no GUS expression pattern. Further characterization of the phenotype and crosses with other floral mutants are underway and will be presented. Reference Sundaresan, V. et al. 1995. Genes & Dev. 9: 1797-1810.

368 ANALYSIS OF THE RESPONSIVENESS OF THE II INTRON AG::GUS TO LOSS OF SAP ACTIVITY

Diana Rigola (1), Detlef Weigel (2), Gerco Angenent (1)

(1) Plant Research International, Business Unit Plant Development and Reproduction, P.O Box 16, 6700 AA Wageningen, The Netherlands. (2) The Salk Institute for Biological Studies, 100010 North Torrey Pines Road, La Jolla, CA 92037, USA

A recessive mutation in the Arabidopsis STERILE APETALA (SAP) causes severe aberrations in inflorescence, flower and ovule development. Several observations provide evidence that SAP, like AP2, LUG, and CLF, is a cadastral gene negatively regulating AG expression (Byzova et al. 1999). In contrast to wild type flowers, sap mutants express AG in the inflorescence meristems and at the earliest stages of flower formation. The role of SAP in specifying the spatial expression of AG is additive to that of the other cadastral genes AP2, LUG, and CLF, because none of these regulators control AG in the inflorescence meristem. To investigate whether the regulation depends on the interaction of SAP with AG sequences, sap mutant plants have been crossed with arabidopsis transgenic plants that express the GUS reporter gene under the control of various AG sequences (Busch et al 1999). GUS expression analysis revealed that SAP does not regulate AG through the large second intron.

Byzova M.V., Franken J., Aarts M.G.M., de Almeida-Engler J., Engler G., Mariani C., Aan Lookeren Campagne M.M., and Angenent G.C.(1999)Arabidopsis SPERILE APETALA, a multifunctional gene regulating inflorescence, flower, and ovule development.Genes & Development 13:1002-1014.

Bush M.A.,Bomblies K., Weigel D. (1999) Activation of a Floral Homeotic Gene in Arabidopsis. Science vol 285 585-587

369 Characterization of enhancer trap markers expressed in the developing fruit
Adrienne Roeder¹, Joel Griffiths¹, Jared Young¹, Sarah Liljegren¹, Heather Cartwright ¹, Yuval Eshed², John Bowman ², Marty Yanofsky ¹
¹Section of Cell and Developmental Biology, University of California at San Diego La Jolla, CA 92093-0116 USA; ²Section of Plant Biology, University of California at Davis, Davis, CA, 95616, USA

As the Arabidopsis gynoecium develops into a mature fruit, cells at the margin between the valves and the replum differentiate to form the dehiscence zone. When the fruit dries, the dehiscence zone cells separate to allow seed dispersal. The SHATTERPROOF (SHP1, SHP2) and INDEHISCENT1 (IND1) genes are required for valve margin and dehiscence zone differentiation, while FRUITFULL (FUL) is necessary for valve cell expansion and differentiation after fertilization. To identify other genes involved in fruit development, especially those which might be regulated by SHP, IND1, or FUL, we have been characterizing enhancer trap markers that show distinct expression profiles in developing fruit. Five enhancer trap lines have valve margin expression patterns, two lines have valve expression, and two lines have other interesting expression patterns in the fruit. The expression patterns of these markers and progress toward identifying the corresponding genes will be presented.

370 Control of Seed Mucilage Cell Development

Scott Sattler and M. David Marks

University of Minnesota

Seed coat mucilage is composed of pectic polysaccharides, which are released from the cell walls of the epidermis when the mature seeds are exposed to water. GL2, TTG and AP2 control seed coat mucilaginous cell development. The hallmark of a mucilage cell is the columella or epidermal plateau, which is a raised structure in the center of the

hexagonal cell. GL2, TTG and AP2 mutants fail to develop columella. To further characterize seed coat mucilage cell development genetically, an India ink screen for mutations that lack mucilage was carried out. The screen resulted in the isolation of 4 new loci involved in seed mucilage development. These new loci have been named MUCilage Modified (MUM). These loci have varying seed coat mucilage cell development. All 4 loci appear to be single gene, recessive mutations. These 4 loci do not affect GL2 expression. GL2 appears to lie upstream of these 4 loci. Three of the loci either lack columella or form abnormal columella. MUM2 mutants form columella and outer cell wall architecture of the mucilage cells resembles wild-type. However, *mum2* seeds completely lack seed coat mucilage. This mutation appears to represent a later step in the development of this cell-type. We are in the process of mapping these loci.

371 Non-autonomous Action of the LEAFY and APETALA1 Transcription Factors

Allen Sessions^{1,2}, Martin F. Yanofsky¹ & Detlef Weigel²

¹Department of Biology, University of California at San Diego, La Jolla, CA 92093, and
²Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037;
weigel@salk.edu

Plant meristems are typically organized into several distinct cell layers. Several floral transcription factors have been shown to signal from layer to layer, but efficient signaling was reported to be layer-dependent, and signaling within layers was always absent or limited.

We have used sector analysis and targeted expression in transgenic plants to investigate whether the floral transcription factors LEAFY and APETALA1 can participate in cell-cell signaling between and within different layers of the floral meristem. We report that LEAFY signals equally well from all layers, and that LEAFY has significant long-range action within layers. Non-autonomous action of LEAFY is accompanied by movement of the protein to adjacent cells, where it directly activates homeotic target genes. In contrast to LEAFY, APETALA1, a structurally unrelated transcription factor that activates an overlapping set of target genes, has only limited non-autonomous effects. These effects appear to be mediated by downstream genes, since activation of early target genes by APETALA1 is cell-autonomous.

Supported by a Department of Energy Fellowship of the Life Sciences Research Foundation (A.S.), and grants from the National Institutes of Health (M.Y.) and the National Science Foundation (D.W., M.Y.).

372 A chromatin immunoprecipitation approach to isolate targets of regulation by AGL15, a MADS-box gene expressed during embryogenesis

Weining Tang*, Cong Zhu*, and Sharyn E. Perry

Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091

AGL15 (for AGAMOUS-Like 15) is currently the only identified member of the plant MADS-box family that is expressed preferentially during embryogenesis. MADS-domain proteins are regulatory factors that are involved in critical developmental decisions in fungi, animals and plants. Evidence to date is consistent with a role for AGL15 as a DNA-binding regulatory factor involved in global aspects of embryo development. In every situation examined to date, AGL15 is present in nuclei when tissue is developing in

an embryonic mode. Structural features of this protein and its accumulation pattern in embryos have been conserved throughout the evolution of angiosperms, arguing for an important and ancient role during this phase of the life cycle. In addition, transgenic Arabidopsis plants that constitutively express AGL15 show a tremendous extension of embryonic potential in culture. To understand AGL15's role in development, genes regulated by AGL15 must be identified. We are using a chromatin immunoprecipitation approach to obtain DNA sequences that bind AGL15 in vivo. This approach uses AGL15-specific antibodies to isolate in vivo formed complexes of AGL15 and DNA. The co-precipitated DNA fragments are recovered, cloned, and the sequence information obtained is used to isolate the putatively regulated genes. Analysis of potential targets of regulation by AGL15 is currently underway. Supported by the University of Kentucky, Dept. of Agronomy, and by the National Science Foundation (Award No. IBN-9984274).

* these authors contributed equally to this work.

373 Surrounding floral organs and ovule structure influence parthenocarpic silique development in the Arabidopsis ffw mutant

Adam Vivian-Smith^{1, 2}, Abed Chaudhury³ and Anna Koltunow²

¹Dept. Plant Science, University of Adelaide, P.M.B, 1 Glen Osmond, 5064, Australia,

²CSIRO, Plant Industry, P.O. Box 350, Glen Osmond, 5064, Australia, ³CSIRO, Plant Industry, G.P.O. Box 1600, Canberra, 2601, Australia

The Arabidopsis ffw mutant is facultatively parthenocarpic and forms a fruit without fertilization. ffw displays a maternal defect that reduces seed set in proximal positions of the silique and a subtle floral phenotype distinct from that in ethylene perception mutants. Parthenocarpic silique development in ffw occurs by mesocarp expansion and division with increased lateral vascular bundle development and ffw is enhanced by emasculation of the surrounding whorls. A unique floral organ interaction was uncovered when double mutants were made with ffw and the aberrant testa shape mutant, resulting in an enhanced parthenocarpic response. To help understand the molecular basis for parthenocarpy we examined silique growth in ffw when hormone perception, ovule and carpel identity functions were removed genetically. Analysis of parthenocarpic silique development in the ffw background using mutants defective in ovule development indicates that a functional nucellus is required for fruit development and that the inner and outer integuments have contrasting roles in controlling fruit development.

374 Role of the F-box Protein UFO in Flower Development

Takuji Wada, Ilha Lee, and Detlef Weigel

Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037;

wada@salk.edu; <http://www.salk.edu/LABS/pbio-w/>

The UNUSUAL FLORAL ORGANS (UFO) gene is an important regulator of homeotic gene expression, as deduced from loss- and gain-of-function studies. However, not all ufo phenotypes are explained by its effects on homeotic gene expression, suggesting that UFO regulates several aspects of floral patterning. One of these is the initiation of petals, and we have shown that UFO acts downstream of AP1 in this process.

The UFO protein contains an F-box domain, which mediates interaction with two Arabidopsis SKP1 homologs, ASK1 and ASK2 (Samach et al., Plant J. 20, 433 [1999]),

suggesting that UFO is part of an SCFUFO complex controlling ubiquitin-mediated protein degradation of unknown targets. Further support for this scenario comes from our observation that an ask1 mutations, which on its own causes only mild floral defects (Zhao et al., *Dev. Genet.* 25, 209 [1999]), can largely suppress the 35S::UFO phenotype, indicating that ASK1 is limiting for the effects of UFO overexpression.

To identify proteins that may be targeted for degradation through their interaction with UFO, we performed several two-hybrid screens with UFO as bait. To circumvent the potential problem that interaction of UFO with yeast SKP1 will preclude detecting an interaction of UFO with prey proteins, we used UFO mutant versions that did not interact with ASK1 in yeast. These included both F-box mutants (P51L62 and P50L62) as well as several other UFO versions with mutations outside of the F box domain. Only the screens with the F-box mutants yielded positive clones, whose interaction could be confirmed in vitro. One protein has an AP2 DNA-binding domain, and the other has homology to a kinase domain of diacylglycerol kinase. Although in vitro interaction of UFO with these two proteins is very weak, the interaction can be enhanced by adding recombinant ASK1, supporting that these proteins interact with a SCFUFO complex in vivo.

This work has been supported by HFSPO and JSPS fellowships (T.W.) and grants from the NSF (D.W.).

375 The role of HEN2 in floral organ identity specification in Arabidopsis

Tamara L. Western and Xuemei Chen

Waksman Institute, Rutgers University, Piscataway, NJ 08854, USA

Floral organ identity in Arabidopsis is determined by the combinatorial activity of A, B and C class homeotic genes. Class A genes alone specify sepals, class A and B give petals, class B and C lead to stamens, while class C alone yields carpels. Class C genes are represented by the "strong" gene AGAMOUS, and the recently identified HUA1 and HUA2, which play overlapping roles in the class C specification of organ identity, determinacy and repression of class A gene activity. In order to identify further genes acting in the class C pathway, an enhancer screen was performed on hua1 hua2 double mutant flowers, which have only weak organ changes (slightly petaloid stamens and bulged carpels). One mutant identified in this screen, hua enhancer DH380 (hen2-1), exhibits homeotic transformation of stamens into petal-sepal mosaic organs and increased bulging and/or lack of carpel fusion in the hua1 hua2 background. This phenotype suggests that both class B and C functions are weakened in the third whorl. In addition, while early expression of the floral organ identity genes seems unaltered, maintenance of their expression appears affected. These data suggest a novel role for HEN2 in the specification of floral organ identity in the third whorl.

376 Stupid Transposon Tricks: Tagging Embryo-Specific Genes in Arabidopsis

Woody, S.T.1, Barton, M.K.1, and Sundaresan, V.2

1Laboratory of Genetics, University of Wisconsin, Madison Madison, WI 53706; 2,

Institute of Molecular Agrobiolgy, National University of Singapore, 59A The Fleming, 1 Science Park Drive, Singapore 118240

We have employed an Ac/Ds transposon tagging approach to identify Arabidopsis genes expressed during embryogenesis. Several lines were recovered in which the GUS reporter

gene of enhancer- or gene-trap transposons is expressed in discrete cell types of the developing embryo. One of those, which we call LENNY, carries an enhancer trap element inserted in a locus (LEN1) that is a member of the EIR/AGR/PIN family of auxin efflux carriers. GUS expression in LENNY is largely confined to the lenticular cell of the hypophysis in globular and transition stage embryos; expression at later stages and after germination is similarly confined to the cells of the QC and surrounding initials. An allelic series of insertions into LEN1 exons was generated by introduction of transposase to remobilize the transposon. RT-PCR assays fail to detect the LEN1 transcript in those derivative lines, but there is no discernable phenotype associated with the knockouts, most likely due to redundant functions encoded by other family members. Interesting changes in GUS expression pattern are observed in those lines. In particular, GUS expression is observed initially throughout young leaf primordia but is subsequently confined to more basal portions of maturing leaves.

The short range transposition pattern of the DsE element was determined by sequencing the flanking genomic sequences in remobilized lines. One of those lines carries a new insertion in a locus with homology to GTP binding proteins. As shown by co-segregation analysis and by the ability to generate revertants in the presence of transposase, disruption of the locus results in arrest of embryogenesis at the transition stage.

377 A novel mutation that alters megaspore and ovule cell fate in *Arabidopsis thaliana*
Wei-Cai Yang, Jian Xu, De Ye, Venkatesan Sundaresan

Laboratory of Plant Molecular Genetics, The Institute of Molecular Agrobiolgy, 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 Kan^R:Kan^S ratio of 1:3, instead of the expected 3:1. From crosses to wild type, we determined that the mutation affects both male and female gametophytes, but is more severe in the female. About 37% of the ovules in the heterozygous plants were aborted. The aborted ovules are arrested at different developmental stages. In some mutant embryo sacs, the polar nuclei fail to fuse, resulting in the abortion of early embryos. More intriguingly, in a fraction of the aborted ovules, instead of a single embryo sac there were four large cells with varying degrees of similarities to embryo sacs. In some cases, an embryo sac at the micropylar end was observed together with additional 3 smaller cells towards the chalazal end. Preliminary data suggest that in these ovules the three non-chalazal megaspores most likely do not undergo apoptosis, but survive and can acquire the ability to form embryo sacs and express a synergid-specific marker. It is also possible that cellularization is affected in the ovule. The mutant line contains a single Ds element inserted downstream of a predicted gene, which we have shown to be expressed in the megaspores following meiosis. Further characterization of this gene and complementation analysis is in progress.

378 Heterodimerization of Floral Organ Identity Proteins

Yingzhen Yang, Laura Fanning, and Thomas Jack

Dept. Biological Sciences, Dartmouth College, Hanover, NH

In the *Arabidopsis* flower, two genes, APETALA3 (AP3) and PISTILLATA (PI) together are necessary and sufficient to specify the development of petals and stamens. Both AP3 and PI encode related DNA binding proteins of the MADS family. In addition to the MADS domain, AP3 and PI also encode a second conserved domain called K domain. The K domain encodes a series of putative amphipathic α -helices, but the function of the K domain has not been determined. Considerable molecular and genetic evidence demonstrates that AP3 and PI function as heterodimer. To identify domains and amino acids that mediate AP3/PI dimerization, we constructed a number of site-specific mutants in conserved amino acids in the K domain. We assayed these mutants for DNA binding (via EMSA) and dimerization (via yeast two-hybrid assay). We also ectopically expressed these mutant constructs in *Arabidopsis* plants under a constitutive promoter to study the in vivo function of the mutated proteins. In addition, we utilized a reverse yeast two-hybrid approach combined with random mutagenesis to identify the critical amino acids mediating AP3/PI heterodimerization. Analysis of these mutants strongly suggests that the hydrophobic amino acids on the putative amphipathic α -helices in the K domain play an essential role in AP3/PI heterodimerization.

379 pistillata-5, a organ identity mutant with defects in a single floral whorl

Yingzhen Yang, Hongjun Xiang, and Thomas Jack

Dept. Biological Sciences, Dartmouth College

In the *Arabidopsis* flower, two genes, APETALA3 (AP3) and PISTILLATA (PI), are necessary and sufficient to specify the identity of petals and stamens. Both AP3 and PI encode related DNA binding proteins of the MADS family. Considerable evidence supports the hypothesis that AP3 and PI function as an heterodimer to direct petal and stamen development. All ap3 and pi mutants to date exhibit defects in both the second and third floral whorls. In this report, we describe the isolation and characterization of pi-5. Unlike all other ap3 or pi mutants, pi-5 exhibits severe defects in the second whorl where sepals develop in place of petals, but third whorl stamens are most often normal. Thus, the pi-5 mutant separates AP3/PI function in the second and third whorls. Analysis of double mutants suggests that pi-5 can specify normal stamen development only in the third floral whorl and only in combination with AGAMOUS (AG), another member of MADS family. Sequencing of pi-5 reveals that Glu125 in PI is changed to Lys. The PI-5 protein exhibits defects in both dimerization with AP3 (via yeast two-hybrid assay) and DNA binding (via EMSA). Together, these data suggests that whorl-specific factors modulate AP3/PI activity in the *Arabidopsis* flower.

380 Adhesion of *Arabidopsis* pollen to stigma cells is mediated by lipophilic interactions

Gregory M. Zinkl, Benjamin I. Zwiebel*, David G. Grier* and Daphne Preuss

Department of Molecular Genetics and Cell Biology and *Department of Physics, The James Franck Institute and The Institute for Biophysical Dynamics. The University of Chicago. 1103 E. 57th Street, EBC 304; Chicago, Illinois 60637

Successful *Arabidopsis thaliana* plant reproduction begins with the binding of pollen to the female stigma cells in the flower. This first step is complete within seconds after contact, before pollen hydration, indicating that adhesion molecules must have the unusual property of functioning in a virtually dry environment. We have developed an

adhesion assay that directly measures the physical force between single pollen grains and stigma cells; in addition, a separate assay examines populations of pollen grains. Stigmas are highly selective since pollen from foreign plants fails to bind with high affinity to *Arabidopsis* stigmas. The lipid- and protein-rich extracellular pollen coat is dispensable for binding, indicating that adhesion molecules localize most likely within the exine walls. As in animal cell adhesion, a change in cell architecture correlates with binding: the stigma surface is remodeled when pollen from *Arabidopsis* and its close relatives binds. Biochemical analyses indicate that this specific, rapid and anhydrous adhesion event is mediated by lipophilic interactions. A genetic approach is underway to isolate pollen adhesion mutants, one of these, *lap1*, is currently being cloned.

381 Absence of floral organ abscission and meristem determinacy in the ethylene-sensitive *Arabidopsis thaliana* mutant inflorescence deficient in abscission (*ida*)

Melinka A. Butenko¹, Abul Mandal², Reidunn B. Aalen¹

¹Division of Molecular Biology, University of Oslo, P.O. Box 1031 Blindern, N-0315 Oslo, Norway. ²Dept. of Natural Sciences, University of Skövde, Sweden

We have identified a T-DNA transformed mutant line of *Arabidopsis thaliana*, named inflorescence deficient in abscission (*ida*), showing no floral abscission and an occasional reversion of floral meristems to inflorescence meristems. Backcrosses and segregation analysis showed that the mutant phenotype is inherited as a single-locus recessive trait. PCR amplification of the *nptII*-marker gene in the T-DNA segregants strongly indicates that the mutation is caused by the T-DNA insertion. Although abscission zone (AZ) cells develop in the *ida* mutant, vivid, turgid floral organs showing no signs of senescence are found attached to full-grown, green siliques. The petals, sepals and stamens dry out completely and still remain attached to the dry septum after the shedding of mature seeds. *ida* seedlings feature the typical triple response of ethylene-sensitive plants indicating that the *IDA* gene is involved in an ethylene-independent developmental pathway controlling floral abscission, and that the *ida* mutant is different from both *etr1* and *ein1* mutants.

Abscission of floral organs is mediated by the induction of endo- β -(1,4)-glucanase (cellulase) genes and other cell wall hydrolytic enzymes. In tomato, up-regulation of *cel5*, a member of the large endo-1,4- β -D glucanase (cellulase) gene family, correlates with in planta flower abscission. Therefore, the spatial expression pattern of the *Arabidopsis* homologue of *cel5*, *celT2H3.5*, was investigated with an in situ hybridisation study performed on wild type and mutant *ida* flowers sampled at given days after pollination. Comparisons of the in situ studies to morphological studies with Ruthenium red confirmed that *celT2H3.5* has an abscission zone-localised expression. However, the pattern of expression did not differ between the wild type and mutant.

PCR techniques have been used to isolate the insert's flanking genomic sequences.

Analyses of these sequences are in progress and will allow us to identify and isolate the entire *IDA* gene, and aid us in determining its role in floral organ abscission.

382 A novel chlorate-resistant mutant, *sds1*, is delayed in leaf senescence

Tanya S. D'Souza, Annie P. Chiang, Chi-Lien Cheng

Department of Biological Sciences, The University of Iowa, Iowa City, IA 52242

A newly isolated chlorate-resistant mutant, *sds1* (sugars and delayed senescence), has been shown to delay the onset of sugar-induced senescence in *Arabidopsis thaliana*. The

sds1 mutant also exhibits a delay in senescence when the detached leaves are treated in the dark or with senescence inducing hormones. An early phenotypical indicator of senescence in Arabidopsis is the yellowing of the rosette leaves, and the sds1 mutant exhibits a delay in this early symptom as compared with the wild type when grown in soil. This delay correlates with a moderate down-regulation of photosynthesis-associated genes (PAGs) and an altered expression of senescence-associated genes (SAGs). The sds1 locus is mapped on the upper arm of chromosome 1. Current progress is underway to fine-map the sds1 locus and to study what role the SDS1 gene product plays in both the sugar and senescence pathways.

383 AGL15 combats "ageing" in flowers and fruits: analysis with molecular markers

Su-Chiung Fang, and Donna E. Fernandez

University of Wisconsin-Madison

AGL15 (AGAMOUS-Like 15) is a member of the MADS domain family of regulatory factors. Although AGL15 is preferentially expressed during embryogenesis, AGL15 is also expressed in leaf primordia, shoot apical meristems and young floral buds, suggesting that AGL15 may play a role during post-germinative development. Transgenic plants that ectopically express AGL15 show delays in the transition to flowering, perianth abscission and senescence (Fernandez et. al., Plant Cell 12:183-197), and fruit and seed maturation. We have used molecular markers to further analyze the effect of ectopic expression of AGL15. Ectopic expression of AGL15 results in a reduction and delay of β -1,4-glucanase (abscission marker) promoter activity in floral organ abscission zones and a reduction and delay of Senescence-associated gene 12 (SAG12) promoter activity in floral organs and silique walls. In seeds, the decline in water content that marks the later stages is delayed. By varying the genetic composition of the embryos, we have shown that this is likely to be a maternal effect. Interestingly, although the rate of desiccation is slower, at least one of the LEA (late embryogenesis abundant) genes is significantly up-regulated in the embryos. Overexpression of AGL15 leads to delays in abscission but where does it act? Is expression in floral organ abscission zones sufficient? To address this question, we generated plants that express AGL15 under the control of a chitinase (abscission zone) promoter. The plants did not show delayed perianth abscission, suggesting that AGL15 expression in other cells contributes to the abscission effect. Funded by the UW-Madison Graduate School, U.S.D.A. (96-35304-3699) and DOE/NSF/USDA Collaborative Program on Research in Plant Biology (DBI 96-02222).

384 EXPRESSION OF AN ABSCISSION-RELATED POLYGALACTURONASE IN Arabidopsis thaliana AND Brassica napus

Zinnia H Gonzalez-Carranza, Jeremy A Roberts

School of Biological Sciences; Plant Science Division; The University of Nottingham.

Sutton Bonington, Loughborough, Leicestershire LE12 5RD

Abscission is a process that involves cell wall degradation, which has been proposed to take place due to the action of hydrolytic enzymes such as polygalacturonase (PG) and β -1,4-glucanase. A fragment of a PG mRNA amplified using a degenerate RT-PCR strategy was used to isolate a full length clone from a cDNA library generated from ethylene-treated Brassica napus leaf abscission zone tissue. Northern analysis revealed that

expression of the PG mRNA accumulates in the presence of ethylene specifically in leaf and flower abscission zones. Expression of the PG can also be detected in adjacent non-separating petiole tissues distal to the site of leaf abscission after prolonged exposure to ethylene. The corresponding PG gene (PGAZBRAN) has been isolated and the promoter analysed for putative regulatory domains. The homologous gene from *Arabidopsis thaliana* (PGAZAT) has been identified and the promoter fused to the GUS reporter gene. An analysis of putative regulatory elements in 1.5kb segment of the promoters has been undertaken. The preparation of some deletion constructs to identify abscission-related domains in the promoter are in process. The temporal and spatial patterns of expression of the PG during cell separation processes will be discussed.

Zinnia H Gonzalez-Carranza. Phone: +44 0115951 6339. Fax: +44 0115951 6334. e-mail: Zinnia.Gonzalez@nottingham.ac.uk

385 Dab5, a new delayed floral organ abscission mutant in *Arabidopsis thaliana*
Joshua D. Lindsey, Shinhan Shiu, Tony Bleecker, and Sara E. Patterson
University of Wisconsin, Madison

Abscission is the active process of cell separation governed by the breakdown of the middle lamella. This is the same process responsible for the leaves falling from the trees in the fall, ripe fruit falling off a tree, and the petals dropping from flowers. To further our knowledge of this event in nature, we have used both reverse and forward genetic approaches to understand the role of genes regulating the process of abscission. Using the T-DNA knockout lines created at Wisconsin, we screened for mutants displaying delayed floral organ abscission. From this screen, we have isolated multiple candidates for the study of floral organ abscission. This poster will present a detailed analysis of the phenotypic, genetic, physiological, and molecular characterization of *dab5*. This mutant is particularly interesting in that it not only has delayed abscission, but it has pronounced apical dominance, dark green rosette leaves, and increased meristem longevity. This work was supported by USDA Grant 98-35301-6764

386 Target-gene analysis of ATHB-1 using a chimeric transcription factor with an inducible function

Takuya Muramoto¹, Mayumi Tsukuda¹, Atsuhiko Oka¹, Satoshi Tabata², Ida Ruberti³, Giorgio Morelli⁴, and Takashi Aoyama¹

¹Institute for Chemical Research, Kyoto University, Japan. ²Kazusa DNA Research Institute, Japan. ³Centro di studio per gli Acidi Nucleici, Italy. ⁴Unita di Nutrizione Sperimentale, Istituto Nazionale della Nutrizione, Italy

ATHB-1 is an *Arabidopsis* transcription factor belonging to homeobox-leucine zipper proteins. Although a transgenic study in tobacco has suggested that it is concerned with the leaf development, its function in *Arabidopsis* has not been determined yet. In this work, we constructed an ATHB-1-derived transcription factor (HD-Zip-1-VG) which was expected to recognize target genes for ATHB-1 and transactivate them depending on the presence of glucocorticoid. In the presence of DEX, a strong glucocorticoid, the transgenic plants expressing HD-Zip-1-VG showed a phenotype of narrow leaves with exaggerated lobes, which was observed also as a severe phenotype of ATHB-1 overexpressor plants. This result indicated that HD-Zip-1-VG recognized same target

genes as those for the authentic ATHB-1. The induction of the transactivating function of HD-Zip-1-VG doesn't require de novo protein synthesis, allowing us to detect the target genes as upregulated transcripts by DEX in the presence of cycloheximide (CHX), an inhibitor of protein synthesis. We carried out a target-gene analysis of ATHB-1 using this induction system in the combination with Arabidopsis genomic DNA clones and DNA microarrays. Biological meanings of obtained candidates for target genes and the role of ATHB-1 in the leaf development will be discussed.

387 A comprehensive genetic approach to understanding cell separation and abscission

Sara E. Patterson and Anthony B. Bleecker

University of Wisconsin, Madison

Processes associated with cell separation occur in a variety of developmental contexts including lateral and adventitious root development, seedling elongation, pollen dehiscence, and pod shatter. Plants also shed entire organ systems such as leaves, flowers, and fruits in a specialized cell separation process known as abscission. Arabidopsis is an excellent model plant for this study due to the variety of mutant collections, the sequencing of the genome, the numerous mapping markers and facile transformation. We have already identified twelve delayed floral organ abscission mutants and are currently cloning several of these genes. We are determining the genetic interactions between these genes as well as known mutants including *etr1-1* and *ein2-1*. We have also measured breakstrength for petal abscission, as well as observed the abscission zone using scanning electron microscopy and light microscopy. In addition, we are using an alternative PCR-based approach in which we are searching for mutants with disruptions in cell wall hydrolytic enzymes. We have identified several "knockouts" and are determining the role of these genes as well as the genetic interactions with our other identified mutants. This broad-based approach will allow us to develop a model for genes regulating abscission and other processes of cell separation.

This work was supported by USDA Grant 98-35301-6764

388 Identification of an Arabidopsis senescence-associated sugar transporter from the Major Facilitator Superfamily

Quirino, B.F. and Amasino, R.M.

University of Wisconsin-Madison

Leaf senescence is an organized, genetically controlled program, that ultimately results in cell death. This last stage of leaf development can be observed as a progressive leaf yellowing due to chlorophyll breakdown. During this period, a number of catabolic pathways increase in activity resulting in the degradation of organelles and cellular macromolecules. In some cases, degradation events are coupled to the formation of transportable molecules. In this manner, nutrients are transported out of the senescing leaf to other parts of the plant such as roots, developing seeds and young leaves. The search for genes involved in the senescence program has not been exhaustive. Therefore, we have used a PCR-based subtractive technique to find new genes that show increased mRNA expression during senescence. One gene identified in this manner is predicted to encode a protein similar to sugar transporters from the Major Facilitator Superfamily of

proteins. Characterization of this gene will help determine whether it is involved in sugar transport during leaf senescence.

389 A WRKY Factor Functions During Certain Cell Death-Related Processes

Silke Robatzek and Imre E. Somssich

Max-Planck-Institut f. Züchtungsforschung, Carl-von-Linne-Weg 10, 50829 Köln, Germany

WRKY6 is a member of the WRKY superfamily of DNA-binding proteins with a single WRKY domain and a leucine zipper. Consistent with its function as a transcription factor, the WRKY6 protein shows nuclear targeting which is mediated by a new bipartite-like NLS.

WRKY6 shows a complex expression pattern with developmental and stress-related components. Expression occurs in all parts of the root, in leaves during early and middle stages of senescence, in flower organs during maturation and in flower organ abscission zones. WRKY6 expression is induced upon wounding, infection with avirulent or virulent bacterial pathogens and upon treatment with elicitor or the signaling substances SA, JA and ethylene. Many aspects of WRKY6 expression are connected with cell death processes often being spatially and temporally restricted to tissues undergoing terminal cell death.

Different regions of the WRKY6 promoter mediate distinct expression behaviors. The promoter fragment up to -218 bp exhibits root specific and leaf senescence expression, the fragment up to -345 bp is sufficient for expression in abscission zones and for induction upon pathogen attack, whereas the fragment up to -726 bp is responsible for flower specific expression. Because of as1-like elements within functional promoter regions, bZIP factors are probably involved in the transcriptional regulation of WRKY6.

The molecular phenotype of two different WRKY6 knock-out mutants effect the expression of SAG12 and PR1 during leaf senescence. In contrast to wildtype plants, these mutants show clear PR1 gene expression in early stages of leaf senescence suggesting that WRKY6 functions as a negative regulator. Consistent with a role in repression, WRKY6 overexpression lines are more susceptible to avirulent pathogens than wildtype plants. In addition to this stress-related phenotype, plant growth is also effected. WRKY6 overexpression lines show a reduction in leaf and rosette size and a faster transition from the vegetative to the reproductive phase. Such data suggest that WRKY6 plays a regulatory role during cell death determination.

390 CHARACTERIZATION OF GLUCOSE INSENSITIVE gin5 AND gin6 MUTANTS, THE ROLE OF ABA IN GLUCOSE REGULATION

Analilia Arroyo Becerra, Francisco Arenas Huertero and Patricia León

Department of Plant Molecular Biology, Institute of Biotechnology, Universidad Nacional Autónoma de México. Cuernavaca Morelos. Av. Universidad 2001, Col. Chamilpa. CP 62250. Tel. 52.73.291667 Fax. 52.73.139988 e-mail: patricia@ibt.unam.mx

Sugars have been shown to have signaling roles in a wide number of metabolic and developmental processes in plants, including responses to environmental stresses. In order to find the components involved in the glucose signaling network, we isolated and characterized a number of Arabidopsis glucose insensitive mutants (gin) based on a

glucose-induced developmental arrest during early seedling morphogenesis. In this work we present the characterization of two of these mutants, *gin5*, and *gin6*. We cloned the T-DNA tagged *gin6* mutant gene and found interestingly, that it corresponds to a new *ABI4* allele. This gene is transcriptionally induced by glucose. Also we have found that the previously isolated *abi4* and *abi5* mutants are glucose insensitive as well, in contrast to *abi1*, *abi2* and *abi3*, which displayed a glucose sensitive phenotype comparable to wild type plants. The characterization of the *gin5* mutant revealed that a glucose-specific accumulation of the ABA hormone is essential for HXK-mediated glucose responses. Consistent with this result, we show that ABA-deficient mutants are also glucose insensitive (*aba*). Exogenous ABA can restore normal glucose responses in *gin5*, *aba* and *abi5* mutants, but not in *gin6* plants. This suggests that the *ABI4/GIN6* putative transcription factor might act downstream to ABA in the glucose-mediated signal transduction cascade. These results provide direct evidence to support a novel and central role of ABA in plant glucose responses. The physiological and molecular characterization of *gin5* and *gin6* mutants will be presented.

391 The Use of Chimeric Enzymes to Identify Regions Involved in Substrate Specificity of Plant Fatty Acid Elongase 3-Ketoacyl-CoA Synthases

Brenda J. Blacklock and Jan G. Jaworski

Miami University

Fatty acid elongase 1 3-ketoacyl-CoA synthase, FAE1 KCS, has been shown to be responsible for the production of very long chain fatty acyl groups (VLCFAs) of seed oils. Despite a high degree of sequence homology between *Arabidopsis thaliana* and *Brassica napus* FAE1, examination of seed oil composition suggests that the enzymes have different substrate specificities. Our objectives are to confirm that the seed fatty acid composition reflects FAE1 KCS substrate specificity and to genetically engineer a KCS with new substrate specificity.

To confirm the substrate specificity of the FAE1 KCSs, the genes were expressed in yeast under control of a galactose-inducible promoter. Both *in vivo* and *in vitro* characterization demonstrated that *A. thaliana* FAE1 KCS prefers 18:1 as a substrate while *B. napus* FAE1 KCS has a greater ability to produce 22:1.

Domain swapping experiments have been conducted to examine the regions of FAE1 condensing enzymes responsible for substrate specificity. Chimeric genes have been engineered, expressed and characterized for activity toward 18:1 and 20:1 substrates. Preliminary results indicated that the amino terminal portion of FAE1 KCS is important to chain length substrate specificity.

392 Characterization of An *Arabidopsis* Geranylgeranyltransferase-I

²Daniela Caldelari, ¹Hasana Sternberg, ²Manuel Rodríguez-Concepción, ²Wilhelm Gruissem and ¹Shaul Yalovsky

¹Department of Plant Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, ISRAEL, ²Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA

Geranylgeranyltransferase-I (GGTase-I) is a heterodimeric enzyme containing an α and a β subunits that modifies proteins with key functions in signaling cascades. GGTase-I recognizes a C-terminal CaaX-box recognition motif in which C designates the

prenylated cysteine, a are usually aliphatic amino acids, and X is almost exclusively a leucine residue. Despite its regulatory importance, cloning of a plant GGT-IB gene and characterization of GGTase-I activity in plants were not reported. The AtGGT-IB gene from Arabidopsis, was cloned using the tomato LeFTA as bait in a yeast two hybrid screen. Sequence comparison showed that the AtGGT-IB protein displays higher homology to its mammalian than to its yeast homologue. Recombinant AtGGTase-I was purified from baculovirus infected insect cells. Kinetic studies, using the purified AtGGTase-I, geranylgeranyldiphosphate, and derivatives of the Petunia calmodulin CaM53 as protein substrates, showed that proteins containing a domain of basic residues proximal to the CaaX box had higher Vmax values. The affinity, however, is determined by the amino acid composition of the CaaX box and remained the same in the absence of the polybasic domain. Moreover, prenylation by GGTase-I was inhibited at high concentrations of a GST-CaaX box fusion protein indicating an inefficient release of the prenylated protein from the enzyme in the absence of the polybasic domain. RT-PCR and protein blot analysis showed that AtGGT-IB is ubiquitously expressed throughout the plant, and that expression AtGGT-IB remains unaltered in era1-2 plants in which the AtFTB gene is deleted. Also, a GFP-CaM53 fusion protein that was transiently expressed in era1-2 leaves was localized in the plasma membrane indicating prenylation of the fusion protein by GGTase-I in the absence of farnesyltransferase.

393 GENETIC MODIFICATION OF OILSEED CROPS TO PRODUCE OILS AND FATS FOR INDUSTRIAL USES

Grace Q. Chen, Thomas A. McKeon, Karen M. Lew, Allan E. Stafford and Jiann-Tsyh
USDA/ARS Western Regional Research Center

The castor bean contains up to 60% oil of unique composition. Up to 90% of the fatty acid content of the oil is 12-hydroxy oleate. As a result, castor oil and products derived from it are used as for bio-based lubricants, paints and coatings, plastics and anti-fungals. The US requires 110 million lbs. Per year, all of which is imported. However, the presence of the toxic protein ricin and highly allergenic storage protein greatly impede the growth and processing of castor in the U.S. Improvement of castor to diminish these hazardous components could expand the domestic production of castor, but improvement of castor has been limited to traits available in germplasm.. We have identified the enzymatic steps that are key to the biosynthesis of castor oil. Several genes for these enzyme have been cloned from castor or other oil seeds and these are being tested to develop transgenic plants that produce an oil with high ricinoleate content. While the model plant Arabidopsis is our initial test system, these genes will be introduced into soybean. We are also taking direct approach to diminish the hazardous components in castor plant by genetic transformation of castor plants.

394 Autoregulation of cystathionine gamma-synthase mRNA stability in response to methionine

Yukako Chiba, Fumiko Kijima, Mari Ishikawa, Jungsup Kim¹, Thomas Leustek¹ and Satoshi Naito

Grad. Schl. Agric., Hokkaido Univ., ¹Cen. Agric. Mol. Biol., Rutgers Univ.

To understand the molecular mechanisms of the regulation of methionine (Met) biosynthesis in higher plants, we adopted a genetic approach utilizing an Arabidopsis

mutant, *mto1* that overaccumulates soluble Met. *mto1* mutants accumulate higher levels of mRNA for cystathionine gamma-synthase (CGS), the enzyme catalyzing the first committed step of Met biosynthesis. Furthermore, exogenously applied Met represses the level of CGS mRNA in WT plants, whereas no such effect is observed in *mto1-1* plants. This suggests that WT plants have a mechanism to repress CGS mRNA accumulation in response to Met, and that this mechanism is impaired in the *mto1-1* plants. Turnover of CGS mRNA in WT and *mto1-1* mutant calli was compared in the presence of a transcriptional inhibitor. CGS mRNA turnover was faster in WT than in *mto1-1* and accelerated by Met in WT only. This showed that the regulation of CGS mRNA accumulation involves a post-transcriptional event. Five independent *mto1* mutations isolated in close proximity within exon1 of the CGS gene each altered the amino acid sequence, suggesting that the exon1 amino acid sequence is responsible for the regulation of CGS mRNA stability. The exon1 region of the CGS gene was fused in-frame to the GUS reporter gene under the control of the CaMV 35S promoter. Constructs carrying WT exon1, *mto1* mutations, or silent mutations were subsequently electroporated into WT protoplasts, which incubated with or without Met. The WT and silent mutants of exon1 were able to down-regulate the GUS activity in response to Met. In contrast, no down-regulation was observed with exon1 containing *mto1* mutations, suggesting that it is the polypeptide of CGS exon1 that is important for this down-regulation. The interaction between WT and *mto1-1* exon1 was investigated by co-transfecting with plasmids carrying WT and *mto1-1* mutant exon1 fused to different reporter genes. Neither the WT nor mutant exon1 affected the reporter activity of the other, suggesting that the exon1 polypeptide acts in cis. We propose a model in which CGS exon1 polypeptide acts to destabilize its own mRNA during translation in response to Met or one of its metabolites.

395 Transgenic overexpression of an ascorbic acid biosynthetic enzyme in *Arabidopsis*

Conklin, Patricia L.

Boyce Thompson Institute for Plant Research, Tower Rd., Ithaca, NY 14853

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide and the hydroxyl radical are generated by metabolic processes, xenobiotic agents, and in response to pathogens in organisms with an aerobic lifestyle. As is the case with all such organisms, plants have the ability to detoxify ROS. L-ascorbic acid (AsA) is one of the best known plant antioxidants. AsA is present in millimolar concentrations in most plant tissues and is a crucial antioxidant and cellular reductant. The ozone-sensitive *Arabidopsis* mutant *vtc1* is deficient in AsA and we have shown that VTC1 encodes a GDP-mannose pyrophosphorylase. This enzyme catalyzes the conversion of mannose-1-P to GDP-mannose, a step in the AsA biosynthetic pathway recently proposed by N. Smirnoff and his colleagues. GDP-mannose is also the activated form of mannose used in protein glycosylation and for the synthesis of cell wall carbohydrates such as mannans, fucose, and L-galactose. Overproduction of GDP-mannose pyrophosphorylase could lead to alterations in cell walls, glycosylated proteins, and/or AsA levels. Transgenic *Arabidopsis* have been generated that over- and underexpress the VTC1 protein. The visual phenotype of these plants as well as their AsA content will be presented.

396 Using confocal microscopy to study plant fatty acid biosynthesis

Gert-Jan de Boer and Chris Somerville

Carnegie Institution of Washington, Dept of Plant Biology, 260 Panama Street, Stanford, CA 94305

A number of enzymes involved in fatty acid synthesis are localized to the microsomal membrane including the integral membrane desaturases, FAD2 and 3, which act on oleic or linoleic acid respectively. Although the genes encoding these enzymes were identified in the early 90's not much is known about their regulation or specific localization. Recently, a growing number of genes from different plant species were identified which encode enzymes such as oleate hydroxylases that are structurally related to the delta-12 desaturase (FAD2). These enzymes are involved in the synthesis of fatty acids, which only accumulate in reserve oils. Overexpression of these enzymes in oilseed crops is an attractive approach generating a renewable source of compounds with a wide variety of industrial applications. To investigate the regulation of the integral membrane desaturases and related enzymes, and to study storage lipid deposition in more detail, we have made translational fusions between a number of enzymes involved in plant fatty acid synthesis, and the green fluorescent protein (GFP). Expression of the Arabidopsis delta-12 desaturase (FAD2):GFP and a Castor oleate hydroxylase:GFP both show endoplasmic reticulum (ER) localization in Arabidopsis. Furthermore, expression of the FAD2:GFP fusion protein in an Arabidopsis *fad2* mutant, restores the fatty acid composition to wild-type levels. This shows that the use of GFP fusion proteins forms an attractive tool to study the regulation of integral membrane desaturases. Another component of plant fatty acid biosynthesis under investigation is oleosin. The endogenous oleosin protein accumulates primarily in tissues producing high levels of storage lipids. Constitutive expression of oleosin fused to GFP in transgenic Arabidopsis shows that the fusion protein is integrated into the oil bodies of storage lipid synthesizing cells. In contrast, transient expression of the oleosin-GFP fusion proteins in Onion epidermal cells showed a fluorescent pattern typical of ER localization. Current experiments are focussed on co-localization of the oleosin and the delta-12 desaturase using different colored fluorescent proteins to specifically visualize the site at which the assembly of the oil bodies takes place.

397 Genetic analysis of autophagy in Arabidopsis thaliana involving the peptide tag APG12

Jed H. Doelling and Richard D. Vierstra

Cellular and Molecular Biology Program and Department of Horticulture, University of Wisconsin-Madison, Madison, WI 53706

Autophagy is the process by which cells degrade intracellular components in bulk within their lysosomes or vacuoles. This process is essential for the survival of yeast and animal cells when subjected to nitrogen deprivation. It was recently shown in the yeast *Saccharomyces cerevisiae* that the formation of autophagic vesicles for delivery into the vacuole is dependent on the proteins APG5 and APG12 in a pathway analogous to ubiquitin conjugation (Mizushima et al., 1998, Nature 395: 395). APG12 serves as a tag and becomes covalently attached to APG5 via an isopeptide bond. Like the ubiquitin pathway, conjugation begins with an activating enzyme (called APG7) and requires a conjugation enzyme (APG10). The molecular details about how this conjugation pathway

contributes to autophagic vesicle formation are unknown; however, the attachment of APG12 to APG5 leads to a change in its subcellular localization. We have searched the Arabidopsis thaliana database for genes encoding orthologs of these 4 APG proteins and have found one or two candidates for each. The nucleotide sequences of cDNAs obtained from these genes indicate that they encode proteins related to their orthologs in yeast and animals. We have also identified two different APG7 T-DNA insertion alleles and are characterizing the phenotype of plants homozygous for each. The mutant plants (like *apg7* yeast) grow normally on high nitrogen (Gamborg or MS) media, but they rapidly become chlorotic when transferred to nitrogen-free media. This indicates a role for APG12 conjugation in the nitrogen cycle in plants.

398 Post-germinative growth and lipid catabolism in oilseeds lacking the glyoxylate cycle

Peter J. Eastmond*, Veronique Germain**, Peter R. Lange*, James H. Bryce**, Steven M. Smith** and Ian A. Graham*

*University of York, Centre for Novel Agricultural Products, Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK. **Institute of Cell and Molecular Biology, University of Edinburgh, The King's Buildings, Mayfield Road, Edinburgh, EH9 3JH, UK.

The glyoxylate cycle is regarded as essential for post-germinative growth and seedling establishment in oilseed plants. We have identified two allelic Arabidopsis mutants, *icl-1* and *icl-2*, which lack the glyoxylate cycle due to the absence of the key enzyme isocitrate lyase (Proc. Natl. Acad. Sci. In Press) These mutants demonstrate that the glyoxylate cycle is not essential for germination. Furthermore, photosynthesis can compensate for the absence of the glyoxylate cycle during post-germinative growth and only when light intensity or day length is decreased does seedling establishment become compromised. The provision of exogenous sugars can overcome this growth deficiency. The *icl* mutants also demonstrate that the glyoxylate cycle is important for seedling survival and recovery following prolonged dark conditions that approximate growth in nature. Surprisingly, despite their inability to catalyse the net conversion of acetate to carbohydrate, mutant seedlings are able to breakdown storage lipids. Results suggest that lipids can be used as a source of carbon for respiration in germinating oilseeds and that products of fatty acid catabolism can pass from the peroxisome to the mitochondrion independently of the glyoxylate cycle. However, an additional anapleurotic source of carbon is required for lipid breakdown and seedling establishment. This can either be provided by the glyoxylate cycle or, in its absence, exogenous sucrose or photosynthesis.

399 Isolation and characterization of lipoxygenase isozymes

Kathrin Fritschea, Ellen Hornunga, Bettina Hauseb, Irene Stenzelb, Claus Wasternackb, Ivo Feussnera

aInstitute of Plant Genetics and Crop Plant Research, D-06466 Gatersleben, Germany;

bInstitute of Plant Biochemistry, D-06120 Halle/Saale, Germany

Lipoxygenases (LOXs) occurring in different forms catalyze the formation of hydroperoxides of polyunsaturated fatty acids. In Arabidopsis a plastid-located leaf-specific AtLOX2 and a root-specific AtLOX1 are known (Melan et al., 1993, Plant Physiol. 101, 441-450; Bell and Mullet, 1993, Plant Physiol. 103, 1133-1137). Here, we

isolated the corresponding cDNAs and characterized the recombinant proteins after expression in *E. coli*. Moreover, we cloned four more full-length cDNAs coding for AtLOX3 to AtLOX6 via PCR based on sequence information of the Arabidopsis data base. Sequence comparisons as well as a phylogenetic tree of all six enzymes will be presented. In addition, we expressed the four new cDNAs in *E. coli* for biochemical characterization. Expression studies using all cDNAs as probes were performed to determine the organ specific expression and to analyze the differential expression upon different stress treatments.

400 Dissecting phloem function using gene and enhancer traps to identify phloem specific gene products

Chiushi Fu¹, Xiaoqin Hu¹, Rob Martienssen², and Daniel R. Bush^{1,3}

¹University of Illinois, ²Cold Spring Harbor Laboratory, & ³ARS-Photosynthesis Research, 196 ERML Urbana, IL

The phloem of the plant's vascular system mediates long-distance transport of sugars and amino acids from sites of primary assimilation to import-dependent "sinks". While this essential function has long been known, it is only recently that we've recognized that the phloem is also a central player in coordinating plant growth by trafficking a variety of developmental and environmental signals. The phloem's sieve elements, which are elongated cells that are linked end-to-end with open pores, form the only tissue system that is continuous throughout the body of the plant. In light of these observations, we are beginning to view the phloem as the plant's version of a simple autonomic nervous system that integrates a wide array of inputs and contributes to the coordinated growth of this multicellular organism. It has been difficult to study the phloem because it is composed of a few minor cell types embedded in complex tissue and organ systems. We have used gene and enhancer traps as a novel approach to identify phloem specific genes that contribute to unique aspects of phloem function. Several transposant lines have been identified with phloem expression patterns that range from organ specific (such as restricted to mature leaves) to global. Our analysis of these plants has shown that several exhibit restricted growth and early flowering. We used TAIL PCR to clone genomic sequences that flank the transposon insertion sites. The tagged genes include transcription factors, receptor kinases, and key metabolic enzymes. We believe these genes play important roles in phloem function.

401 What Does Sugar Do, and How Does It Do It?

Susan I. Gibson, Ron J. Laby, Donggiun Kim, Jennifer To and Kelly Biddle

Department of Biochemistry & Cell Biology, Rice University, Houston, TX USA 77005-1892

Soluble sugar levels have been postulated to play an important role in the control of a variety of plant developmental and metabolic pathways. However, exactly which pathways are regulated, at least in part, by soluble sugar levels remains unclear. In addition, little is known about the signal transduction pathways by which sugar levels exert their effects. Our lab is taking several approaches to address these issues. First, "DNA chip" analysis was used to screen ~ 10,000 Arabidopsis ESTs to identify those that are sugar-regulated at different stages in development. These experiments indicate that

expression of a significant percentage of Arabidopsis genes is affected by soluble sugar levels and are helping to elucidate which pathways may be sugar regulated. We have also isolated Arabidopsis mutants that show an impaired ability to sense and/or respond to sugar. These mutants were isolated by screening mutagenized seeds for those that are 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 seeds germinate, but develop into seedlings that show little or no shoot development. Media-shift experiments indicate that wild-type Arabidopsis seedlings lose sensitivity to the inhibitory effects of high sugar concentrations within ~ 48 hours after the start of imbibition. These results, together with lipid analyses and other experiments, suggest that sugar-mediated inhibition of early seedling development may be related to inhibition of seed storage lipid breakdown. Additional experiments are being performed to test this hypothesis.

Nineteen sugar-insensitive (*sis*) mutants have been isolated using the above screen. Seven *sis* mutants carry defects in the *ABI4* gene, while two other *sis* mutants are allelic to the *aba2* mutant. The *sis1* mutation is in the *CTR1* gene, which encodes a component of the ethylene signal transduction pathway. Other *sis* mutants can germinate in the presence of an inhibitor of gibberellin biosynthesis. These results suggest that sugar response pathways may interact, directly or indirectly, with several phytohormone response pathways.

Supported by Department of Energy Biosciences Program Grant #DE-FG03-98ER20300.

402 Identification of glucosinolate mutants based on isothiocyanate bioactivity

Clayton D. Grubb, Heidrun B. Gross, and Steffen Abel

University of California, Davis

Glucosinolates are a diverse class of sulfur- and nitrogen-containing secondary metabolites that are mainly found in members of the Brassicaceae. Upon tissue disruption, glucosinolates are rapidly hydrolyzed by myrosinases to unstable intermediates that spontaneously rearrange to isothiocyanates, thiocyanates, or nitriles, compounds that have a variety of biological activities. Natural isothiocyanates derived from glucosinolates are potent chemopreventive agents that favorably modulate carcinogen metabolism by inhibiting metabolic carcinogen activation and/or by inducing carcinogen-detoxifying enzymes. Methylsulfinylalkyl isothiocyanates are potent selective inducers of Phase 2 detoxification enzymes such as quinone reductase. Members of the Cruciferae family including Arabidopsis synthesize methylsulfinylalkyl glucosinolates. We have adopted a colorimetric bioassay for quinone reductase activity in Hepa 1c1c7 murine hepatoma cells as an efficient tool to rapidly monitor methylsulfinylalkyl glucosinolate content in Arabidopsis leaf extracts. Using various ecotypes and mutant plants defective in the synthesis of methylsulfinylbutyl glucosinolate (glucoraphanin), we have demonstrated that ecotype Columbia is a rich source of Phase 2 enzyme inducers and that glucoraphanin accounts for most of the quinone reductase inducer potency of Columbia leaf extracts. We have optimized leaf extraction conditions and the quinone reductase bioassay to allow for screening of large numbers of plant extracts in a molecular genetic approach to dissecting glucosinolate biosynthesis in Arabidopsis. To date we have screened 3,000 M2 plants (ecotype Columbia) and have verified by HPLC

analysis eight mutant lines with significantly altered glucosinolate content and composition. Identification of structural and regulatory genes required for glucosinolate synthesis is the long-term goal of our research. Such genes will be used as molecular tools to elucidate biological roles of glucosinolates in plants and to metabolically engineer crops with enhanced cancer-preventive potential.

403 The *ref2* gene is required for developmental regulation of phenylpropanoid secondary metabolism in *Arabidopsis*

Matthew R. Hemm, Max O. Ruegger¹, and Clint Chapple

Department of Biochemistry, Purdue University, West Lafayette, IN 47907; ¹current address: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268

Sinapate esters are end products of the phenylpropanoid pathway in *Arabidopsis* and other members of the Brassicaceae. The accumulation of these esters is developmentally regulated. Sinapoylcholine is the primary sinapate ester found in seeds and is thought to be a seed storage form of both sinapic acid and choline. During germination, sinapoylcholine is converted to sinapoylmalate, which accumulates in the cotyledons. After germination, sinapoylmalate is synthesized *de novo* in leaves, where it is thought to serve as a UV protectant. All sinapate esters share a blue-green fluorescence under UV light, which can be observed *in vivo*. This *in vivo* fluorescence was used to identify the *ref2* mutant. *ref2* mutants accumulate reduced levels of sinapoylmalate in leaves but have wild-type levels of sinapoylcholine in seeds. The *ref2* mutation also affects the biosynthesis of lignin, another end product of the phenylpropanoid pathway. The lignin polymer deposited in *ref2* stems has a significantly lower percentage of syringyl subunits; however, the total lignin levels are unaffected. The reduction of both sinapic acid esters and syringyl lignin supports the role of REF2 in the regulation of phenylpropanoid secondary metabolism during vegetative development. Other pleiotropic phenotypes of *ref2* include a hypersensitive flower-wounding response and the accumulation of novel sinapic esters in seeds. The REF2 gene is currently being isolated by positional cloning.

404 The sugar-insensitive 2 (*sis2*) Mutant Germinates in the Absence of Gibberellin

Lydia C. Hoot, Donggiun Kim, Jennifer P.C. To, and Susan I. Gibson

Rice University

Sugar levels help regulate a number of plant developmental processes. Little is known about the mechanisms by which plants respond to sugar levels. Characterization of plant sugar response mutants will help us to understand these mechanisms.

A sugar-insensitive mutant (*sis2*) was isolated from EMS-mutagenized seeds based on its ability to germinate and form true leaves on high levels (0.3M) of exogenous glucose. Wild-type seeds can germinate under these conditions, but cotyledons turn purple and do not expand; leaves do not develop. This is not purely an osmotic effect because wild-type seeds can germinate and form true leaves on 0.3M sorbitol. In addition to being insensitive to glucose, *sis2* is also insensitive to the inhibitory effects of 2mM mannose on seed germination and early seedling development. To determine whether other sugar responses are defective in *sis2*, the effects of exogenous sugar levels on chlorophyll and anthocyanin production were tested. The *sis2* mutant exhibits wild-type production of chlorophyll and anthocyanin on exogenous sugar. This suggests that the sugar transport mechanism in *sis2* is unaffected by the mutation, as otherwise all sugar responses would

presumably be altered. As work by several labs has indicated connections between sugar and phytohormone response pathways, we characterized the responses of *sis2* to phytohormones. The *sis2* mutant exhibits normal responses to ethylene and to abscisic acid, but an altered response to gibberellin during germination. Unlike wild-type seeds, *sis2* seeds can germinate on paclobutrazol, an inhibitor of gibberellin biosynthesis. We are currently examining *sis2* responses to gibberellin at other stages of plant development, including time to flowering and rate of stem elongation. Surprisingly, neither exogenous gibberellin applied to wild-type seeds, nor the *spy3* mutation (a mutation conferring a constitutive response to gibberellin), promote normal seedling development on high sugar concentrations. The *sis2* mutation has been mapped to chromosome 1, 11.4 cM away from *nga111*, as a first step towards cloning the *SIS2* gene.

This research was supported by Department of Energy Biosciences Program Grant #DEFG03-98ER20300.

405 Characterization of two cytochrome P450s involved tryptophan secondary metabolism

Anna K. Hull, Rekha Vij, Neeru Gupta, Brenda Levy and John L. Celenza
Dept. of Biology, Boston University, 5 Cummington St., Boston, MA 02215

Tryptophan and its indolic biosynthetic intermediates serve as precursors for secondary metabolites with diverse functions. These include the plant growth regulator indole-3-acetic acid (IAA) as well as the plant defense compounds camalexin and indole glucosinolates. Although several biosynthetic pathways have been proposed for the production of secondary indolic compounds most of the genes encoding the enzymes involved in these pathways have not been cloned.

We have identified two *Arabidopsis* cytochrome P450s, *CYP79B2* and *CYP79B3*, that can both catalyze the conversion of tryptophan (Trp) to indole-3-acetaldoxime in an *in vitro* assay. This reaction is the first step in pathways proposed for the biosynthesis of both IAA and indole glucosinolates. Plants overexpressing *CYP79B2* are resistant to 5-fluoroindole, 5-methyltryptophan and 5-methylanthranilate which suggests that *CYP79B2* is involved in Trp metabolism *in vivo*. In addition, the *CYP79B2* mRNA is pathogen induced in a manner similar to other Trp biosynthetic genes.

To study the expression pattern of *CYP79B2* and *CYP79B3* in plants, we have made transgenic plants carrying a *CYP79B2* or *CYP79B3* β -glucuronidase (GUS) reporter. These reporters both show GUS activity in the root tip, the base of lateral roots, and the vascular tissue of the shoot. Both reporters were designed as translational fusions and contain the first ~ 45 amino acids of *CYP79B2* or *CYP79B3* which are predicted to encode a chloroplast transit peptide. As anticipated, both reporters are plastid localized which is the site of tryptophan and IAA biosynthesis.

Current work focuses on the further biochemical analysis of *CYP79B2* and *CYP79B3* and determination of the role of these enzymes in Trp metabolism. To address these questions as well as the question of redundancy, mutations in these two genes have been constructed and are being characterized.

406 New insights into phenylpropanoid metabolism provided by characterization of ferulate 5-hydroxylase, a cytochrome P450-dependant monooxygenase

John M. Humphreys, Matthew R. Hemm, and Clint Chapple

Department of Biochemistry, Purdue University, West Lafayette, IN 47907

Lignin is the second most abundant biopolymer on Earth. In addition to its physiological roles as a structural element of secondary cell walls and as a component of plant defense response, lignin has economic importance to the paper, forestry and forage crop industries. Precursors for lignin are synthesized by the phenylpropanoid pathway.

Although the enzymes and genes of this pathway have been studied for several decades, the cloning of the ferulate 5-hydroxylase (F5H) gene from *Arabidopsis* has provided the first opportunity to study this key enzyme in lignin biosynthesis. F5H is a cytochrome P450-dependant monooxygenase known to catalyze the hydroxylation of ferulate to 5-hydroxyferulate; however, our studies indicate that the enzyme also uses coniferaldehyde and coniferyl alcohol as substrates. Unexpectedly, the K_m values measured for the latter two substrates are three orders of magnitude lower than that measured for ferulic acid. This suggests that in lignifying tissues, F5H may function later in the phenylpropanoid pathway than was originally proposed. To further test the model that multiple syringyl monomers may be derived from their guaiacyl counterparts by hydroxylation and subsequent methylation, a coupled assay using recombinant F5H and native or recombinant *Arabidopsis* caffeic acid/5-hydroxyferulic acid O-methyltransferase was devised. In this coupled assay, all three F5H substrates were converted to their corresponding sinapyl derivatives, indicating that the necessary enzymes required for these alternate pathways are present in *Arabidopsis*. Taken together, these data suggest that the previously accepted pathway for lignin biosynthesis is likely to be incorrect. To test our pathway model outside *Arabidopsis*, genes from other plants have been cloned and the enzymatic properties of these P450s are being compared. In addition, a collection of F5H alleles from EMS-mutagenized *Arabidopsis* has also been identified. These mutant F5H alleles will be used to help identify catalytically and structurally important amino acid residues of the enzyme.

407 The FLAKY POLLEN gene which is essential for production of pollen coat sterols

Sumie Ishiguro, Miho Yamada, Natsuko Kageyama and Kiyotaka Okada

Department of Botany, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

Pollen recognition is a crucial step in fertilization in higher plants. In plants with dry stigmas, such as *Arabidopsis*, this process takes place at the very first stage of pollination on the surface of stigmas. Previous studies have implicated that the pollen surface components have essential roles in the cell-cell communication process between pollen grains and stigmatic papillae.

We have isolated a male-sterile mutant designated flaky pollen (flk), based on the deficiency of its pollen to germinate on the stigmatic papillae. flk cannot produce a normal pollen coat. This prohibits pollen recognition by the stigma, which would normally lead to the hydration of the pollen by the pistil. flk pollen restores fertility, however, when pollination is allowed to take place under high humidity conditions. Biochemical analysis of the flk pollen revealed that it was severely lacking sterol esters. The FLK gene encodes 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMG-CoA synthase), an enzyme that functions in the initial steps of the mevalonate pathway in

isoprenoid biosynthesis. In higher plants, this pathway is responsible for the production of cytosolic terpenoids, including sterols. Current data from the Arabidopsis genome project suggest that the FLK is the only gene encoding this enzyme. However, the flk mutant shows no detectable abnormality other than the defect of pollen function. Moreover, the mRNA of FLK is highly expressed in the flower buds but not detected in the leaves in wild type plants. These findings suggest that the requirement of the mevalonate pathway is restricted in some tissues such as tapetum for pollen coat production. Also it is possible that the "non-mevalonate" pathway, which is responsible for the terpenoid production in plastids, is highly active in almost all tissues in Arabidopsis and can compensate for the lack of mevalonate pathway.

408 Ecotype Association And Bacterial Expression Assays Of Glucosinolate Side Chain Modification Enzymes Identified By Map Based Cloning

Lambrix, V.M., Kliebenstein, D.J., Reichelt, M., Gershenzon, J. and T. Mitchell-Olds
Max Planck Institute for Chemical Ecology, Carl Zeiss Promenade 10, 07745 Jena, Germany

Glucosinolates are phytochemicals found predominantly in the order Capparales. Tissue disruption brings together glucosinolates and myrosinases, glucosinolate specific hydrolyzing enzymes, to yield a variety of breakdown products. These breakdown products have been shown to influence human nutrition and taste preferences, seed germination, and plant defense mechanisms. The diverse biological activities are dependent upon differential side chain modifications of glucosinolates. To better understand the biological function of these side chain modifications, we utilized natural variation to map base clone genes regulating side chain modifications. This has led us to three 2-oxoglutarate dependent dioxygenases (2-ODD's) involved in the modification of glucosinolate side chains.

Map base cloning of enzymes responsible for the accumulation of methylsulphinyl, alkenyl and hydroxy-propyl glucosinolates led us to a small gene family of three tightly linked 2-ODD's. Sequence and expression based analysis of 35 different ecotypes has provided evidence for the specific activity of each enzyme. Results suggested that Alk/Ohp2 converts methylsulfinyl glucosinolate to alkenyl, while Alk/Ohp3 converts the 3-methylsulfinyl to 3-OHpropyl. The oxidation of methylthiol to methylsulfinyl may be carried out by Alk/Ohp1, however the evidence is not as compelling. Enzyme assays have provided further evidence for Alk/Ohp2 and Alk/Ohp3 reactions.

Mapping natural variation for the conversion of 3-butenyl to 2-OH3-butenyl in crosses between four Arabidopsis ecotypes places the enzyme responsible for this conversion in the same region. Additionally, we have been able to show a correlation between the expression of a functional candidate enzyme and conversion of the 3-butenyl in lines that do not normally accumulate the 3-butenyl precursor. Non functional candidate enzymes did not convert 3-butenyl. The candidate gene has been expressed in *E. coli*. Results from the ecotype association studies and enzyme assays will be discussed.

409 Map-Based Cloning of the VTC2 (Vitamin C 2) Locus

Irena M. Levin, Susan R. Norris, Jeyanthi Ramamoorthy and Robert L. Last
Cereon Genomics, 45 Sidney Street, Cambridge, MA 02139

Vitamin C (L-ascorbic acid) is a potent antioxidant and cellular reductant involved in cell wall expansion and cell division. A novel pathway for ascorbate biosynthesis was recently proposed (Wheeler and Smirnoff, *Nature* 393: 365-369, 1998); however, the existence of additional biosynthetic pathway(s) remains a topic of debate. To identify genes involved in plant ascorbate levels, Conklin and co-workers identified a collection of ascorbate reduced mutants (*vtc*, vitamin C) in *Arabidopsis* (*Genetics*, 154: 847-856, 2000). Our work has focused on the *vtc2* mutant, which was previously mapped to the lower arm of chromosome IV (*Genetics*, 154: 847-856, 2000). This poster will describe the use of map-based cloning to narrow the *VTC2* containing region to 19 kb. Sequencing this region from the mutant and subsequent comparison to the wild-type sequence (GenBank Accession # AL035440) identified a single open reading frame which differed between wild-type and *vtc2*. The identified open reading frame encodes a gene of unknown function. In addition to complementation studies, biochemical and molecular characterizations of the *VTC2* gene are in progress.

410 Plant Genomes contain Multiple Aldehyde Dehydrogenase Genes

Feng Liu^{1,3}, Tsui-Jun Wen², David S. Skibbe^{1,4}, Jun Cao^{1,3}, Xiangqin Cui^{1,3}, Carl Simmons⁶, Robert B. Meeley⁶, Henry Weiner⁷, and Patrick S. Schnable^{1,2,3,4,5}
Departments of 1Zoology & Genetics, 2Agronomy; 3Interdepartmental Genetics Program; 4Molecular, Cellular and Developmental Biology Program, 5Center for Plant Genomics; Iowa State University, Ames, IA 50011. 6Pioneer Hi-Bred International, Johnston, IA

Plant genomes contain multiple genes that encode Class 1 and Class 2 ALDHs. For example, the maize genome contains two genes that encode Class 1 (*rf2a* and *rf2b*) and Class 2 (*rf2c* and *rf2d*) ALDHs. In contrast, although the *Arabidopsis* genome also contains two Class 1 ALDH genes (*ataldh1*, and *ataldh3*), evidence to date indicates that contains only one Class 2 ALDH gene (*ataldh2*). The physiological functions of these ALDHs are in most cases not yet known. However, genetic analyses have established that the Class 1 mitochondrial (mt) ALDH encoded by the maize *rf2a* gene is required for normal pollen development. The *rf2a* gene has been expressed in *E. coli* and the corresponding recombinant protein purified to homogeneity. Kinetic analyses revealed that this mtALDH can catalyze the oxidation of a diverse collection of aldehyde substrates. K_m s for aliphatic aldehydes are in μM range, which is similar to the low K_m mammalian mtALDHs. The *E. coli* expressed *rf2b*, *rf2c*, *ataldh1* and *ataldh2* genes also exhibit ALDH activity in crude cell extracts when acetaldehyde and glycolaldehyde are used as substrates. Preliminary analyses have revealed that the second maize mtALDH (encoded by the *rf2b* gene) has a higher K_m for acetaldehyde than does the *rf2a*-encoded mtALDH. The plant ALDHs exhibit other differences in substrate preferences. For example, the *rf2a*, *rf2c* and *ataldh2* genes (but not the *rf2b* and *ataldh1* genes) can complement an *E. coli* *aldh* mutant that requires lactaldehyde dehydrogenase activity. Plant genomes also contain a number of putative Class 7 fatty aldehyde dehydrogenase genes. Reverse genetic approaches are being used to "knock out" the Class 1, 2 and 7 plant ALDH genes in an effort to determine their physiological functions in maize or *Arabidopsis*.

411 NITRATE-REGULATION OF FLOWERING TIME

Irene Loef, Mark Stitt and Anne Krapp

Institute of Botany, University of Heidelberg, INF 360, D-69120 Heidelberg, Germany

Nitrate is one of the major nutrient for plants, but its abundance in soil varies. Therefore plants have to adjust to changes in nitrogen availability. Early physiological studies have shown that nitrate uptake and nitrogen assimilation is induced by re-supplying nitrate to nitrogen-starved plants. More recently, it was demonstrated that nitrate itself triggers multiple responses, like induction of nitrate and ammonium assimilation, organic acid production and inhibition of starch synthesis, thus resulting in reprogramming of primary C/N metabolism. Nitrate signaling is also important for plant development. It has been shown, that nitrate is directly involved in the regulation of root architecture and adjustment of the shoot/root ratio. We now present first evidence that nitrate influences flowering time in *Arabidopsis thaliana* regardless of the overall nitrogen status. Plants were grown on solid media supplied with glutamine in order to maintain a high nitrogen status despite the nitrate concentration. Plant growth as a whole was not affected by adding 1 or 10 mM nitrate, but on 10 mM nitrate flowering was retarded by 5-7 days compared to 1 mM nitrate. This effect was not due to pH changes. We are currently investigating the flowering-retarding effect of nitrate in mutants in nitrogen-assimilation and flowering. Plants were grown on solid media supplied with glutamine in order to maintain a high and constant organic nitrogen status independent of the nitrate supply. Overall plant growth (e.g. relative growth rate, rosette-size and number of leaves) was unchanged on 1 and 10 mM nitrate. However, flowering was delayed by 5-7 days in high nitrate compared to low nitrate conditions, and this was not due to pH changes. We are currently investigating the nitrate-regulated changes of flowering-time using mutants that are affected in nitrogen-assimilation or flowering.

412 Functional genomics of *Arabidopsis* secondary metabolite glycosyltransferases
Messner, B.; Schindler, E.; Thulke, O.; Wegener, S.; Schaeffner, A.R.; Sandermann, H.Jr.

GSF Research Center for Environment and Health, Institute of Biochemical Plant Pathology, D-85764 Neuherberg, Germany

Plant secondary metabolite glycosyl transferases (PSM-GT) comprise a huge family of enzymes involved in glycosylation of a large variety of compounds. The carbohydrate moiety is conjugated to hydroxyl, amino, or sulphhydryl groups thus tagging, activating, or inactivating the parent molecule. In xenobiotic metabolism of certain herbicides or fungal toxins glycosylation is a crucial step in detoxification preceding export from the cytosol. Glycosylated precursors are involved in e.g. lignin biosynthesis or pigmentation. Signaling molecules like phytohormones are inactivated or transported via glycosylated forms. These few examples point to the broad impact of glycosylation in plant metabolism. The *Arabidopsis* genome encodes about 100 PSM-GTs.

In order to investigate the functional roles of the members this large gene family we study their spatial and developmental expression patterns and their inducibility by different treatments using a DNA microarray harboring gene specific probes. In addition, selected individual genes from different branches are recombinantly expressed to investigate their substrate specificity. Interestingly, initial tests of three enzymes reveal a rather broad specificity comprising a range of endogenous substrates like kaempferol, anthocyanidines, hormones and xenobiotic substances like chlorophenols or herbicide

metabolites. In the future we plan to complement these analyses by studying the physiological consequences of knock-out mutants on the metabolite pattern for natural and xenobiotic substrates and other phenotypic parameters.

413 Cytochrome P450s from the CYP79 family catalyzes the conversion of amino acids to aldoximes in the biosynthesis of glucosinolates
Mikkelsen, M. D., Wittstock, U., Hansen, C. H. and Halkier, B. A.
Plant Biochemistry Laboratory, Dep. of Plant Biology, Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Copenhagen, Denmark
Glucosinolates are natural plant products found in the Capparales order, which includes agriculturally important crops such as rape and many vegetables. Glucosinolates and their hydrolysis products exhibit a wide range of biological effects as e.g. flavour compounds, attractants and deterrents for herbivorous insects and as defense compounds. In *Arabidopsis* more than 23 glucosinolates have been identified. These represent the major classes of glucosinolates, the aliphatic, aromatic and indole-glucosinolates, thus making *Arabidopsis* an ideal model system for the study of glucosinolates. Glucosinolates are related to cyanogenic glucosides as both compounds are derived from amino acids and have aldoximes as intermediates. Cytochrome P450s from the CYP79 family have been shown to catalyze the conversion of both aliphatic and aromatic amino acids to their corresponding oximes in the biosynthesis of cyanogenic glucosides. The nature of the enzymes catalyzing the conversion of amino acid to oxime in the biosynthesis of glucosinolates has been subject to much debate. Biochemical studies in the Brassicaceae family have indicated that flavin containing monooxygenases convert the aliphatic amino acids and homophenylalanine to their aldoximes. Plasma membrane peroxidases have been shown to convert tryptophan to indole-3-acetaldoxime, a metabolite in the biosynthesis of indoleglucosinolates and of indole acetic acid. However, we have identified several CYP79s from *Arabidopsis* which catalyze the conversion of aliphatic, aromatic and indole amino acids to their corresponding oximes. By altering the expression level of these CYP79s in *Arabidopsis* we have found a direct correlation between the expression of the CYP79s and the glucosinolates which they produce. This shows that cytochrome P450s catalyze the formation of aldoximes in glucosinolate biosynthesis, that the biosynthesis of glucosinolates has evolved based on a 'cyanogenic' predisposition, and that CYP79s from glucosinolate producing species have evolved beyond the cyanogenic predecessors by being able to metabolize substrates unknown from the biosynthesis of cyanogenic glucosides.

414 Adenosine kinase deficiency is associated with developmental abnormalities and reduced transmethylation
Moffatt BAa, Stevens Ya, Allen Ma, Snider Ja, Pereira La, Martin McCaffrey La, Summers PSb, von Schwartzberg Kc
aDept. of Biology, University of Waterloo, ON; bDept of Biology, McMaster University ON., cInstitut f. Angewandte Botanik, Hamburg
Adenosine kinase (ADK) which catalyzes the salvage synthesis of AMP from adenosine and ATP is encoded by at least two genes in *Arabidopsis*. The protein products of these two genes are 92% identical. Based on their Kms both ADK1 and ADK2 have a high affinity for adenosine (0.3-0.5 μ M) as well as for cytokinin ribosides (3-5 μ M). Each

gene is constitutively expressed with the highest steady-state mRNA levels being found in stem and root.

A set of transgenic lines deficient in ADK activity have been created by over-expression of the sense or antisense strand of the ADK1 cDNA. The most deficient lines which have approximately 7% of the ADK activity found in wild-type plants are small, with rounded, wavy leaves and a compact bushy appearance. Due to the lack of elongation of the primary shoot, their siliques extend in a cluster from the rosette. Hypocotyl and root elongation are reduced as well. Plants with 15-30% ADK activity have a more intermediate phenotype whereas those with 50% ADK activity have a wild-type morphology. There is a direct correlation between the level of methylesterified pectin in seed mucilage and ADK activity, as monitored by ruthenium red staining or direct assay. Reduced methylesterification is consistent with the ADK-deficient lines being affected in methyl recycling and transmethylation.

415 Isolation and characterization of *uns2* mutant of *Arabidopsis* showing unusual sugar response

Masa-aki Ohto¹, Shingo Hayashi^{1,2}, Yasuko Furukawa¹, and Kenzo Nakamura^{1,2}
¹Dept of Develop. Biol., Natl. Inst. for Basic Biol., Okazaki, 444-8585 Japan, ²Grad. Sch. of Bioagric. Sci., Nagoya Univ., Nagoya, 444-8601 Japan

When wild-type *Arabidopsis* plants were cultured on a medium with sugars, flowering time were delayed and the chlorophyll content of leaves were increased by the increased level of sugars in the medium (Ohto et al, 9th this meeting, 1998, Onai et al, 10th this meeting, 1999). In an effort to understand the role of sugar signalling in the floral transition or leaf development of *Arabidopsis*, we have isolated mutants from our enhancer-tagged mutant lines which showed early flowering and/or low chlorophyll content in leaves on the medium with high levels of sugars and also showed unusual regulation of sugar-responsive genes in leaves. A recessive mutant, *uns2*, was isolated by such screening. The *uns2* showed early flowering and low level of chlorophyll in leaves regardless of the growth conditions, either in vitro with sugars or on soil. In *uns2*, regulation of sugar-inducible genes, such as *Atb-Amy* and *CHS*, was altered. Cloning of DNA region flanking to the T-DNA showed that T-DNA was inserted into *HLS1* gene, an ethylene-responsive gene. Phenotypes in several alleles of *hls1* with respect to flowering, the chlorophyll content and sugar-regulated expression of *Atb-Amy* and *CHS* were similar to those in *uns2*, indicating that *UNS2* turned out to be *HLS1*. In *hls1*, the sugar-regulated expression of other sugar-responsive genes was also altered. To examine the subcellular localization of *HLS1* protein, transgenic plants carrying *HLS1::cMyc* have been prepared and the results with the transgenic plants will be presented. The role of *HLS1* in the sugar-responsive gene expression will be discussed.

The authors are grateful to Dr. Joseph Ecker at Univ. of Pennsylvania for his kind gift of *HLS1* cDNA and seeds of several alleles of *hls1* and seeds of *35S::HLS1* plants. References; Lehman, A., Black, R. and Ecker, J., *Cell*, 85: 183-194 (1996)

416 Mevalonate pathway or non-mevalonate pathway: Which pathway do plants use for GA and ABA biosynthesis?

Kazunori Okada, Hiroshi Kawaide, Bunshiro Endo, Tomohisa Kuzuyama*, Haruo Seto* and Yuji Kamiya

RIKEN Wako-shi, Saitama, Japan, 351-0198, *Institute of Molecular and Cellular Biosciences, University of Tokyo, Bunkyo-ku, Tokyo, Japan, 113-0032

Mevalonate pathway or non-mevalonate pathway: Which pathway do plants use for GA and ABA biosynthesis?

Kazunori Okada, Hiroshi Kawaide, Bunshiro Endo, Tomohisa Kuzuyama*, Haruo Seto* and Yuji Kamiya RIKEN, Wako-shi, Saitama, Japan, 351-0198, *Institute of Molecular and Cellular Biosciences, University of Tokyo, Bunkyo-ku, Tokyo, Japan, 113-0032.

Isopentenyl diphosphate (IDP), which is a general precursor of plant isoprenoids, can be synthesized by two different pathways, namely the mevalonate (MVA) pathway in cytosol and the non-MVA pathway in plastids. Since enzymes catalyzing early steps of gibberellins (GA) and abscisic acid (ABA) biosynthesis localize in plastids, the plastidial non-MVA pathway is thought to be a major pathway to supply the precursor for these plant hormones. However, we still do not know how the non-MVA pathway is involved in the GA and ABA biosynthesis. To address this question, we have cloned the genes related to the non-MVA pathway, and examined their functions, expression patterns, and phenotypic effects by silencing of the genes.

So far, two genes involved in the first two steps of the non-MVA pathway have been reported in Arabidopsis. Recently, Escherichia coli 2-C-methyl-D-erythritol-4-phosphate (MEP) cytidyltransferase that catalyzes the third step of the non-MVA pathway to produce cytidyl-MEP has been reported. We have cloned its Arabidopsis homolog (AtMEPCT) and purified His-tagged AtMEPCT protein produced in E. coli. The recombinant protein showed cytidyl-MEP transferase activity. We generated antisense plants for the AtMEPCT gene. The transgenic plants showed pale green albino-like phenotype which was also seen on the plant treated with non-MVA pathway specific inhibitor, fosmidomycin, suggesting that the AtMEPCT gene affects the production of pigments such as carotenoids and chlorophylls through the plastidial non-MVA pathway. Since AtMEPCT protein has a possible transit signal peptide in its N-terminal region, this protein is probably localized in plastids and may be involved in the GA and ABA biosynthesis along with pigment biosynthesis.

417 The sugar-insensitive 3 (sis3) Mutant Exhibits Decreased Sensitivity to the Inhibitory Effects of Sugars on Early Seedling Development

Donna L. Pattison, Donggiun Kim, Susan I. Gibson

Department of Biochemistry & Cell Biology, Rice University, Houston, TX 77005-1892 USA

Sugar is known to be an important metabolite in plant processes, but little is known about the role of sugar as a signaling molecule. Sugar has been shown to be involved in regulating metabolism, gene expression, and development. High levels of sugars delay seed germination of Arabidopsis thaliana and block the development of true leaves. Interestingly, high levels of glucose inhibit starch breakdown and germination of cereal seeds (Thomas and Rodriguez, 1994). It is, therefore, possible that high glucose levels inhibit lipid breakdown and germination in the oil seed plant, Arabidopsis, in a manner similar to the way cereal plants regulate the breakdown of starch. To test this hypothesis, lipid analysis studies are now in progress.

The *sis3* mutant of *Arabidopsis* was isolated from a T-DNA tagged line by screening for plants that can develop true leaves on 0.3 M sucrose media. *sis3* plants are insensitive to the inhibitory effects of sugar on germination and true leaf development. The mutant, however, is not defective in all sugar responses. *sis3* shows a wild-type increase in anthocyanin levels and chlorophyll levels when grown on high sugar media, therefore, it is unlikely that *SIS3* is a sugar transporter. Unlike other known sugar response mutants, *sis3* does not appear to be defective in its response to gibberellin, abscisic acid, or ethylene. The mutation may, therefore, be directly linked to sugar sensing and control rather than indirectly connected through another signaling pathway. The *sis3* gene maps near the bottom of chromosome 1 close to the *nga280* marker. Since the mutant is tagged, the GenomeWalker Kit (Clontech) will be used to clone the gene. Understanding how plants respond to sugar is important, because ultimately it may lead to the engineering of crop plants that shuttle more of their sugar products to the parts of the plant that are used for food, thus increasing crop yield.

Supported by Department of Energy Biosciences Program Grant #DE-FG03-98ER20300

418 The *Ble* gene as a reporter for the activity of a carbon dioxide sensitive promoter in *Chlamydomonas reinhardtii*

Steve Vincent Pollock and James Vant Moroney

Louisiana State University

The carbon dioxide concentrating mechanism (CCM) allows algae to concentrate CO₂ in the vicinity of Rubisco to decrease the rate of photorespiration under low CO₂ conditions. The CCM is not present under high CO₂ conditions and is present under low CO₂ conditions. One of the best characterized components of the CCM in the green alga *Chlamydomonas reinhardtii* is the periplasmic carbonic anhydrase. It is encoded by the gene *Cah1*. To elucidate the components of the CO₂ signal transduction pathway of the CCM, we have linked the *Cah1* promoter to the *Ble* gene to make the construct pCABLE. The *Ble* gene confers Zeocin resistance in *C. reinhardtii*. When cells transformed with pCABLE are grown under low CO₂ and Zeocin on agar they are able to grow. When pCABLE transformants are grown under high CO₂ and Zeocin they die. Thus the *Ble* gene fused to the CO₂ responsive promoter of *Cah1* can be used as a reporter for the CO₂ signal transduction pathway of *C. reinhardtii*. Random insertional mutagenesis and UV mutagenesis are now being used to select for mutants that are insensitive to changes in the level of CO₂. One type of mutant could lead to constitutive expression of *Ble* under high CO₂ and low CO₂ conditions. We are hopeful that by using this system we will identify some of the components involved in the CO₂ signal transduction pathway.

419 Biochemical and Molecular Characterization of *Arabidopsis* N-Myristoyltransferase

Qungang Qi (1), Raju Rajala (2), William Anderson (1), Chao Jiang (1), Kevin Rozwadowski (1), Gopalan Selvaraj (1), Rajendra Sharma (2), and Raju Datla (1)

1. Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, SK S7N 0W9, Canada, and 2. Department of Pathology and Saskatoon Cancer Center, University of Saskatchewan, Saskatoon, SK S7N 4H4, Canada

Myristoyl-CoA:protein N-myristoyltransferase catalyzes the co-translational addition of myristic acid to the amino-terminal glycine residue of a number of signaling proteins of diverse functions. To obtain better insights into the biochemical and molecular properties of this important enzyme in plants, we have isolated a full-length *Arabidopsis thaliana* cDNA encoding NMT (AtNMT1), the first described from higher plants. This AtNMT1 cDNA clone has an open reading frame of 434 aa and predicted molecular mass of 48,706 Da. The deduced aa sequence of AtNMT1 is closely related to that of mammalian NMTs, and the residues suggested to be essential for enzyme function are conserved. Analyses of Southern blots, genomic clones, and database sequence suggest that *A. thaliana* genome contains two copies of the NMT gene which are present on different chromosomes and distinct genomic organizations. The recombinant AtNMT1 enzyme showed high catalytic efficiency for the AtCDPD6 and Fen kinase peptides. The AtNMT1 gene expression profile indicated ubiquity in roots, stem, leaves, flowers and siliques (1.7 kb transcript and 50 kDa immunoreactive polypeptide) but a greater level in the younger tissues. NMT activity was also evident in all these tissues. The majority of the immuno-reactive NMT polypeptide was associated with the ribosomal fraction. The results presented in this study provide the molecular evidence for plant protein N-myristoylation and a mechanistic basis for understanding the role of this protein modification in plants.

420 From sequence to function: the challenge of characterizing putative glycosyltransferase genes in *Arabidopsis*

Todd Richmond, Norbert Sprenger, and Chris Somerville

Carnegie Institution of Washington, Department of Plant Biology

The effort to sequence the entire *Arabidopsis* genome has proven to be a treasure trove for plant molecular biologists and cell wall researchers. A large superfamily of cellulose synthase (CesA) and cellulose synthase-like (Csl) genes has been identified in *Arabidopsis*, consisting of at least six subfamilies and over forty different genes. Homologs of many of these genes have been found in a wide variety of plant species, from mosses to trees. Sequence analysis indicates that these genes have conserved protein domains found in processive glycosyltransferases. Our laboratory is taking a reverse genetic approach to determine the function of several of these families of putative glycosyltransferases. We will discuss our progress in answering four important questions: where and when are these genes expressed, what is their enzymatic function, and what is their importance in the biosynthesis of the plant cell wall.

421 Intron Features Required To Elevate Gene Expression

Alan B. Rose

Molecular and Cellular Biology, University of California, Davis, CA 95616

Introns are known to stimulate the expression of many plant genes. The magnitude of this intron-mediated enhancement (IME) can be as large as 1,000-fold, but is more commonly in the range of 2- to 10-fold and is typically larger in monocots than dicots. Unlike enhancer elements, introns increase mRNA accumulation without affecting the rate of transcription, and only do so when located within transcribed sequences in their normal orientation. While the mechanisms of IME are not fully understood, these results suggest

that introns act post-transcriptionally by facilitating mRNA maturation or stabilizing the nascent RNA.

The 110 nt first intron of the Arabidopsis PAT1 gene mediates a 5-fold increase in mRNA accumulation from a PAT1:GUS fusion in single-copy transgenic plants. The PAT1 intron 1 features required for IME were explored by generating a series of altered introns. To determine whether specific intron sequences are necessary, four small overlapping deletions which together span the intron were made. All four of these introns elevated PAT1:GUS mRNA accumulation as much as did the wild-type intron, demonstrating that individual intron sequences are entirely dispensable for IME. To test if splicing is required, the intron was made unspliceable either by point mutation in the 5' splice site or by deletions that rendered the intron too small to be spliced. All three unspliceable "introns" were able to increase mRNA accumulation, showing that splicing is not required for IME.

All of these mutant introns might still contain the features necessary to be recognized as introns, even if they cannot be spliced, and this recognition could be required for IME. Spliceosome assembly onto introns could promote an association with other stabilizing aspects of mRNA maturation such as capping, polyadenylation, or nuclear export. A possible connection between intron recognition and IME is currently being explored by mutating the PAT1 intron 1 structural elements found in most dicot introns: the 5' and 3' splice sites, potential branchpoints, and intron U-richness. Also, the ability of introns from several genes to elevate PAT1:GUS expression are being compared to determine which characteristics are shared by those introns best able to stimulate expression.

422 Genetic Analysis of Sulfate Uptake and Assimilation

Nakako Shibagaki^{1,2}, Jeffrey McDermott², Toru Fujiwara¹, Hiroaki Hayashi¹, John P. Davies²

¹ Dept. Appl. Biol. Chem., Univ. Tokyo, Yayoi, Bunkyo, Tokyo, Japan ² Dept. Botany, 459 Bessey Hall, Iowa State Univ., Ames IA 50011-1020

Plants are primary producers of sulfur-containing organic molecules such as cysteine, methionine, glutathione and phytochelatins. Sulfur is imported into root cells as sulfate through sulfate transporters. Sulfate is then transported into plastids where it is activated, reduced and used in the synthesis of cysteine, the main precursor of other sulfo-organic compounds. In sulfur deficient soils, plants increase sulfate uptake and assimilation. We are investigating sulfur metabolism in plants by isolating and characterizing mutants of *Arabidopsis thaliana* that are defective in sulfate uptake or assimilation. To identify these mutants we have screened for plants that are resistant to the toxic sulfate analogues selenate (Se) and chromate (Chr). Seeds were germinated on medium lacking sulfate but containing concentrations of Se and Chr that inhibit root growth of wild-type plants. We screened for plants able to grow roots. We reasoned that mutants that have deficiencies in sulfate transport, assimilation or the ability to induce these processes under sulfur-limiting conditions might assimilate less Se and Chr and have longer roots.

Here, we describe two Se-Chr resistant mutants. The mutant 32-4 was isolated from an EMS mutagenized population and 1-8 from a population of T-DNA transformed plants. These mutants also show cotyledon expansion on medium containing 200 μ M selenate, a concentration that inhibits growth of wild-type plants. Sulfate uptake experiments show

that the mutants are deficient in sulfate transport when grown in either sulfate-replete or sulfate-deficient media.

Genetic analysis indicates that these mutants contain allelic recessive nuclear mutations. The lesion in 32-4 has been mapped to a 400 kbp region on the lower arm of chromosome I containing two of the nine sulfate transporter genes in Arabidopsis. Analysis of the T-DNA in the 1-8 mutant indicates that the promoter region of one of these sulfate transporter genes is disrupted; the coding sequences of the other may be defective also. Thus, the mutation in 32-4 appears to be in one of these sulfate genes. The sequence of these sulfate transporters from the 32-4 mutant is being analyzed.

423 The SNG2 locus encodes a serine carboxypeptidase-like protein that functions in Arabidopsis secondary metabolism

Amber M. Shirley, Colleen M. McMichael¹, and Clint Chapple

Department of Biochemistry, Purdue University, West Lafayette, IN 47907
current address: DuPont Central Research and Development, Biochemical Sciences and Engineering, Experimental Station, Wilmington, DE 19880

Serine carboxypeptidases have been traditionally assigned roles in protein processing and turnover because of their ability to cleave C-terminal peptide bonds. In plants, serine carboxypeptidase-like (SCPL) proteins have often been suggested to have similar roles because of their co-incident expression in tissues actively involved in proteolysis. We recently reported on an SCPL protein from Arabidopsis that catalyzes a transesterification reaction in sinapate ester secondary metabolism, indicating that SCPL proteins may have evolved much broader substrate specificity and catalytic versatility. Presently, the Arabidopsis Genome Initiative has identified 25 SCPL proteins encoded in the Arabidopsis genome, for which the *in vivo* function of only one of these proteins has been determined. Here we report another example of an SCPL protein in secondary metabolism, identified using an Arabidopsis mutant sinapoylglucose accumulator 2 (*sng2*) that accumulates sinapoylglucose in seeds instead of sinapoylcholine. Homozygous *sng2* seeds accumulate increased levels of choline but have significantly decreased activity of sinapoylglucose:choline sinapoyltransferase. The developmentally regulated accumulation of sinapate esters in the *sng2* mutant is essentially undisturbed except that the biosynthetic precursor sinapoylglucose accumulates to wild-type sinapoylcholine levels. The SNG2 locus was cloned by a combination of map-based methods and a candidate gene approach. The present report and recent examples of other SCPL proteins with diverse catalytic functions suggests that some of the other Arabidopsis SCPL proteins may function in reactions other than cleavage of peptide bonds.

424 Blocking Histone Deacetylation Induced Pleiotropic Effects on Plant Gene Regulation and Development

Lu Tian and Z. Jeffrey Chen

Texas A&M University

Histone acetylation and deacetylation plays an essential role in eukaryotic gene regulation. Reversible modifications on core histones are catalyzed by intrinsic enzymes, histone acetyltransferase (HAT) and histone deacetylase (HD). In general, histone deacetylation is related to transcriptional gene silencing, while acetylation correlates with

gene activation. In eukaryotic organisms that employ both DNA methylation and histone modifications, histone deacetylases are recruited by DNA methyl binding proteins (e.g., MeCP2, MBD2), DNA methyltransferase (Dmmt1), or sequence-specific DNA binding proteins to silence genes. To elucidate the role of histone deacetylation in plant gene regulation and development, we have produced transgenic plants using antisense *Arabidopsis* histone deacetylase (AtHD1) gene, a homolog of RPD3 global transcriptional regulator in yeast. Expression of antisense AtHD1 caused dramatic reduction in endogenous AtHD1 transcription, resulting in accumulation of acetylated histones notably tetra-acetylated H4. Reduction in AtHD1 expression and AtHD1 production and changes in acetylation profiles are associated with various phenotypic and developmental abnormalities, including early senescence, ectopic expression of silenced genes, suppression of apical dominance, homeotic transformation, heterochronic shift towards juvenility, and flower defects. Some of the phenotypes could be attributed to ectopic expression of flower specific genes (e.g., SUPERMAN) in vegetative tissues. These results suggest that AtHD1 is a global regulator that controls gene expression during development through DNA-sequence independent or epigenetic mechanism in plants as in other eukaryotes. In addition to DNA methylation, epigenetic regulation involving histone modifications may be a general regulatory mechanism responsible for plant plasticity and variation in nature.

425 Regulation of glutamine synthetase by asparagine - role of HY5 transcription factor

Mary E. Williams and Monica J.A. Lee

Harvey Mudd College, Biology Department, 301 E. 12th St., Claremont, CA 91711

Glutamine synthetase (GS) expression is sensitive to levels of free amino acids. In particular, light-induction of GS activity in the shoot is reduced by exogenous Asn, which accumulates under conditions of high nitrogen. To elucidate the mechanism by which Asn regulates GS activity, we screened for Asn-resistant *Arabidopsis* mutants. Through this screen, we identified a novel mutant allele of the gene encoding the transcription factor HY5. Plants carrying this mutant HY5 gene are hindered in their light-induction of GS in the shoot, suggesting a role for the HY5 transcription factor in expression of GS.

426 A gene family with similarity to DNA-J like proteins is involved in gravitropism

Kanokporn Boonsirichai, Elizabeth S. Rosen, and Patrick H. Masson

Laboratory of Genetics. University of Wisconsin-Madison. 445 Henry Mall, Madison, WI 53706

A small gene family in *Arabidopsis thaliana* has been identified on the basis of sequence similarity to the ARG1 gene. This gene has previously been shown to be necessary for wild type response to gravity, but not for phototropism or root-growth responses to auxin or other plant hormones (Sedbrook et al. 1999. PNAS USA 96:1140-1145). The other two members of the gene family, ARL1 and ARL2, show highly conserved sequence throughout several functional domains including the DNA J domain, the predicted coiled-coiled domain, and the transmembrane domain. Mutations have been identified in both of these genes, and the physiological characterization of some of these mutants has shown phenotypes similar to those of *arg1* mutants.

427 Mutations affecting light regulation of nuclear genes encoding chloroplast glyceraldehyde-3-phosphate dehydrogenase

ChuiSien Chan and Ming-Che Shih

Department of Biological Sciences, University of Iowa, Iowa City, Iowa 52242

We have used a negative selection scheme to isolate mutants that are defective in light activation of the nuclear gene (GAPA) that encodes A subunit of chloroplast glyceraldehyde-3-phosphate dehydrogenase. We have transformed a Col/adh1-2 line, which contains a null mutation in the ADH gene, with a transgene carrying two consecutive reporter genes, ADH and GUS, each driven by a GAPA promoter. Screening of 20,000 transgenic M2 plants allowed us to identify four mutants that are defective in light induction of both reporter genes. Northern blot analyses indicated that these mutants are also defective in light-regulated transcription of the endogenous GAPA and GAPB genes. In addition, our data showed that these mutants also reduced mRNA levels of several other nuclear genes that encode Calvin cycle enzymes, including chloroplast triosephosphate isomerase (cplTPI) and phosphoglycerate kinase (cplPGK). These mutants are therefore tentatively named ucc mutants (for underexpressor of Calvin cycle genes). Our data indicated that these mutants have normal hypocotyl lengths under different light spectra. In addition, our preliminary data indicated that mutations in these UCC genes do not affect light-regulated expression of LHC (chlorophyll a/b binding proteins) genes. These results suggest that discrete signaling steps are involved in light activation of Calvin cycle genes and LHC genes in Arabidopsis.

428 ELF3 Gates Input to the Circadian Clock

Michael F. Covington^{1,3}, Xing-Liang Liu^{1,4}, Steve A. Kay², and D. Ry Wagner^{1,4}

¹Institute of Molec. Biology, Univ of Oregon

²Dept of Cell Biology, The Scripps Research Institute (TSRI)

³Present Address: Dept of Cell Biology, TSRI

⁴Present Address: Agritope, Inc., Portland, OR

The Arabidopsis circadian clock is involved in photoperiodic induction of flowering. How the clock measures photoperiod information and relays it to the flowering pathway(s) is unknown. EARLY FLOWERING3 (ELF3), a novel gene, is implicated in light perception, circadian clock, and flowering pathways. Besides flowering early, the elf3 mutant has an elongated hypocotyl, long petioles, and is pale compared to Wild-Type (WT)¹. elf3 mutants have been shown to be arrhythmic in constant light conditions² and defective in circadian rhythm responses following a red light pulse in dark-grown seedlings³.

An elf3-promoter::luciferase (elf3::luc) reporter system was designed and used with other circadian markers, elf3 mutant alleles, and ELF3 overexpressing plants to describe the regulation of ELF3 and assess its role in these three pathways.

We demonstrate that ELF3 acts under high-intensity red light as part of a gating mechanism for input to the clock by showing: 1) waveform and level of ELF3 expression is dependent upon photoperiod; 2) the circadian clock remains intact in plants overexpressing ELF3; 3) ELF3 overexpression lengthens the period of the clock at higher red light fluence rates; and 4) the presence of ELF3 is sufficient and necessary for entrainment, even in a dysfunctional circadian system.

The circadian nature of ELF3's expression coupled with the clock's dependence upon ELF3 for input/entrainment allow the clock to be responsive to resetting stimuli only during specific phases of the circadian cycle. This suggests that ELF3's role in the circadian system is to gate light input to the clock.

1M. T. Zagotta, et al., *Plant J.* 10, 691-702 (1996).

2K. A. Hicks, et al., *Science* 274, 790-792 (1996).

3S. L. Anderson, et al., *Plant Cell* 9, 1727-1743 (1997).

429 Multiple Heme Oxygenases are Required for Proper Phytochrome-Regulated Development

Seth J. Davis, Seong Hee Bhoo, Adam Durski, and Richard D. Vierstra
University of Wisconsin-Madison

A complex network of receptors and signal-transduction pathways mediate plant responses to changing light environments. The best-characterized component of this network is the phytochrome family of photoreceptors that regulates plant morphogenesis and growth. To help understand how the phytochrome holoprotein is assembled, we have identified and characterized the first committed step in phytochrome-chromophore biosynthesis. Previously, we isolated the *Arabidopsis thaliana* HY1 gene, whose mutation severely reduces holo-phytochrome levels. It encodes a higher-plant heme oxygenase (HO), designated AtHO1, which is responsible for catalyzing the reaction that opens the tetrapyrrole ring of heme to generate biliverdin IX α . However, because *hy1* mutant plants regain some photomorphogenesis and holo-phytochrome as they mature, it is likely that other HOs are present. Using AtHO1 as a query, we identified three additional HOs in *A. thaliana*, referred to as AtHO1-AtHO4. We have started investigating the role of AtHO2, the most divergent member of the HO gene family, through the characterization of a *ho2-1* mutant isolated through a reverse-genetic strategy. In this mutant, both apo- and holo-phytochromes can be detected. Like *hy1* plants, *ho2-1* plants display a range of phytochrome-deficient phenotypes, including a decrease in chlorophyll accumulation, a reduced growth rate, a developmental acceleration of flowering time, and a partial defect in deetiolation. These data confirm the physiological importance of multiple HOs in phytochrome-chromophore synthesis and holo-phytochrome production. Our results suggest that the biosynthesis of the phytochrome chromophore is an additional mechanism that regulates phytochrome action.

430 REGULATION OF ABSCISIC ACID (ABA) BIOSYNTHESIS IN ARABIDOPSIS: CHARACTERIZATION OF THE 9-CIS-EPOXYCAROTENOID DIOXYGENASE GENE FAMILY

Wen-Tao Deng, Bao-Cai Tan and Donald R. McCarty
PMCB Program, University of Florida, Gainesville, 32611

ABA regulates seed dormancy and germination, stomatal aperture, and gene expression in response to environmental stresses. In plants, ABA is synthesized from carotenoids localized on chloroplast membranes. The key regulatory step of ABA biosynthetic pathway is the oxidative cleavage of 9-cis-epoxycarotenoids. The limiting enzyme, 9-cis-epoxycarotenoid dioxygenase (NCED) is encoded by a complex gene family. We isolated five NCED-like genes in *Arabidopsis*, designated AtNCED1 to 5, which showed strong

homology to VP14 and other NCED's in tomato and bean. Northern analysis revealed a distinctive tissue specific expression pattern among AtNECDs in different tissues and upon stress. Transgenic plants overexpressing AtNCED2 showed a phenotype of fewer lateral roots and male sterility. AtNCED2 promoter-GUS transgenic lines showed GUS staining at the base of mature lateral roots, a narrow ring of cells in root cap, and pericycle cells surround lateral root initials. IAA treatment induced lateral root formation and also strongly induced GUS staining, suggesting that ABA is involved in the lateral root initiation. Chloroplast import assay revealed that AtNCED2 is partly localized in stroma and partly bound to thylakoid membrane, whereas AtNCED3 and AtNCED5 localized exclusively to stroma. AtNCED1 is not imported into chloroplasts. These data suggest that accessibility to epoxy-carotenoids by AtNCEDs may play an important role in the regulation of ABA biosynthesis.

431 Engineering the Phytoremediation of Arsenic Contaminated Soil

Om Parkash Dhankher, Barry P. Rosen¹ and Richard B. Meagher

Department of Genetics, University of Georgia, Athens, GA ¹Department of Biochemistry and Molecular Biology, Wayne State University, Detroit, MI

Arsenic is an extremely toxic heavy metal pollutant that adversely effects the health of millions of people worldwide. In Bangladesh and India, more than a million people suffer from arsenic-induced skin lesions, hyperkeratoses and/or cancer due to arsenic exposure. The currently available technologies for soil and water clean up are extremely expensive and are impractical on the scale that is needed. Phytoremediation (plant based remediation) is a promising new technology that utilizes plants to degrade, metabolize or detoxify heavy metals and organic contaminants in soil and water. Heavy metals, unlike organic contaminants can not be metabolized or degraded by plants. An alternative approach would be to use plants to extract and concentrate arsenic above ground. Bacteria have evolved a mechanism to resist arsenic by reducing arsenate ($\text{AsO}_4\text{-III}$) to arsenite ($\text{AsO}_3\text{-III}$) and eliminate arsenite via a membrane transporter. The oxyanion arsenate, an analogue of phosphate, is the most prevalent form of arsenic in soils and has the potential to be translocated into plants along with phosphate. The reduced oxyanion arsenite ($\text{AsO}_3\text{-III}$) is comparatively more toxic than arsenate ($\text{AsO}_4\text{-III}$) and is a highly thio-reactive reagent that is unlikely to be mobile in any system. Therefore, one potential phytoremediation strategy is to express a bacterial arsenate reductase and sulphur-rich arsenite-binding peptides in the above ground tissues. In order to convert arsenate to arsenite, the *E.coli*arsC gene encoding arsenate reductase was overexpressed in the leaves of *Arabidopsis thaliana* using a leaf specific promoter. Transgenic plants showed symptoms of arsenite poisoning in leaves but not in roots when grown on media containing $75\mu\text{M}$ sodium arsenate. At higher concentrations, transgenic plants died whereas wild type plants remained healthy. Western analysis of transgenic plants confirmed that arsenate reductase is expressed only in leaves and not in roots. Speciation of arsenic species in some transgenic lines showed slightly elevated level of arsenite. We are currently in the process of co-expressing sulphur-rich arsenite-binding peptides in *Arabidopsis* and results will be presented in the near future.

432 New pieces in the phytochrome signaling puzzle

Christian Fankhauser¹ and Joanne Chory²

¹ Département de Biologie Moléculaire Université de Genève, 30 quai E. Ansermet, 1211 Genève 4. Plant Biology Laboratories, The Salk Institute for Biological Studies, La Jolla CA-92037

The phytochromes (phy) sense red and far-red light. In higher plants they are encoded by a small gene family, PHYA-PHYE in *Arabidopsis thaliana*. Phytochromes covalently bind a chromophore and display well-understood spectral properties. Biochemical mechanisms for the initial events in phytochrome signaling are beginning to emerge. Both the stability and the Ser/Thr protein kinase activity of Phytochrome A are light regulated. Moreover both phyA and phyB translocate from the cytoplasm to the nucleus in a light dependent fashion.

Despite much recent progress in the characterization of the photoreceptors we still know relatively little about signaling components acting downstream. Several phytochrome signaling intermediates have been cloned during the past 2 years but based on the complexity of the light response and the number of genetic loci affected in those processes many remained to be identified.

The characterization of new mutants affected in red/far-red sensing will be presented. *rsf1* (reduced sensitivity to far-red light) is a recessive mutant with a phenotype similar to a weak phyA allele, suggesting that it represent a positively acting factor in phyA signaling. *rsf1* mutants have elongated hypocotyls in far-red and blue light but no defects in red light sensing. However, phyA dependent anthocyanin accumulation in far-red light is not affected in this mutant, suggesting this gene affects only a subset of phyA responses.

dsr1 (decreased sensitivity to red) mutants are recessive, they respond normally to blue and far-red light but hypocotyl elongation in red light is poorly inhibited. *dsr1* plants are also slightly pale and flower early most notably in short-days. All these phenotypes are very similar to those of phyB mutants. Co-segregation of the T-DNA resistance marker and the mutant phenotype in 70 plants indicates close linkage of the mutation and the T-DNA.

Further characterization and cloning of new phytochrome signaling mutants should allow us to understand the molecular mechanisms of phytochrome mediated light perception.

433 Dissection of the light-signal transduction pathways that regulate Elip expression in *Arabidopsis*

Orit Harari-Steinberg and Daniel A. Chamovitz

Department of Plant Sciences, Tel Aviv University, Ramat-Aviv 69978, Israel

The expression of light regulated genes in plants is controlled by different classes of photoreceptors that act through a variety of signaling molecules. In the de-etiolation process, the Elip (early light Inducible protein) genes are among the first to be induced. To elucidate and integrate the light signal transduction pathways that regulate Elip expression, we are studying the regulation of the two Elip genes, Elip1 and Elip2, in *Arabidopsis thaliana*, taking advantage of the genetic tools available for studying light signaling in *Arabidopsis*. Using two independent semi-quantitative RT-PCR techniques, we have found that the light regulation of each Elip gene is different. Red, far-red and blue lights induce Elip. Phytochrome A and phytochrome B are involved in this signaling, but not the cryptochrome or the phototropin photoreceptors. This implicates a

novel blue-light receptor in Elip regulation. Zeaxanthin is not the chromophore of this blue-light receptor. The transcription factor HY5 has an essential role in controlling the light induction of Elip. Furthermore, Elip is induced in a light independent fashion in the *hy5* mutant by heat shock.

434 COP1 interacts with HY5 and STO proteins through a novel motif
Magnus Holm, Christian Hardtke, Rachele Gaudet and Xing-Wang Deng
Dept of MCDB, Yale University

COP1 is a WD40 domain containing protein that acts as a repressor of photomorphogenesis in Arabidopsis. COP1 interacts both genetically and physically with HY5, a positive regulator of photomorphogenesis. The interaction is mediated by the N-terminal half of HY5 and the WD40 repeats in COP1. Recent results suggests that COP1 negatively regulates the abundance of HY5 protein in the dark through proteasome dependent degradation. In an effort to learn more about the function of COP1, in particular the role of the WD40 domain, we have used two complementary approaches. 1) Based on the X-ray structures of WD40 domain proteins, we substituted 5 amino acids in the COP1 WD40 domain, that are likely to mediate protein-protein interactions. The five amino acid substituted COP1 proteins were introduced into the null allele *cop1-5*, to investigate potential partial phenotype rescue. All five COP1 proteins rescue the lethality of *cop1-5*, however, they confer different responses to both light quality and light intensity. 2) Five novel COP1 interacting proteins (CIPs) were identified in a yeast two-hybrid screen, all of which interact with the WD40 domain of COP1. The CIPs interact differentially with the amino acid substituted COP1 proteins but two of them, STO and a STO homolog (STH), interact in a pattern similar to that of HY5. Both HY5 and the STO/STH proteins contain an 11 amino acid motif, located in the N-terminal half of HY5 and in the C-terminus of the STO/STH proteins. The motif is negatively charged with a highly conserved VP pair at the carboxy end. VP to AA substituted STH and HY5 proteins are unable to interact with COP1 in yeast. Moreover an E to K substitution in the motif in HY5 abolishes the interaction with wild-type COP1 but allows an interaction with a K to E substituted COP1 protein. The same K to E substituted COP1 protein is unable to interact with the wild-type CIP proteins, suggesting that these two amino acids directly interact. Furthermore, over-expressed VP to AA substituted HY5 protein is able to rescue the *hy5* phenotype in plants, but show a dramatic reduction of HY5 degradation in the dark.

435 Influences of development and environmental conditions on the expression of the wax-specific condensing enzyme CUT1 in Arabidopsis
Tanya Hooker, Sabine Clemens, Anthony Millar and Ljerka Kunst
Department of Botany, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Epicuticular waxes are complex mixtures of long-chain hydrocarbons and their derivatives produced in plants from very long chain fatty acids (VLCFAs). The Arabidopsis thaliana gene CUT1 encodes a VLCFA condensing enzyme required for the production of epicuticular wax in bolting stems. Since epicuticular waxes are secreted over the surfaces of all aerial parts of the plant, and environmental conditions often influence wax deposition, we have examined the expression pattern of CUT1 in

Arabidopsis at different developmental stages and under different environmental conditions. Analysis by RNA blot and in situ hybridization showed that CUT1 was highly expressed in epidermal cells of all aerial parts of the plant, but not in roots. CUT1 expression was initiated at very early stages of development, in seedlings and inflorescence meristems, and was highest in rapidly expanding tissues, but transcripts were still abundant in mature tissues. As tissues aged, CUT1 expression was reduced. Light was required for CUT1 expression, and its expression was increased by cold, salt and drought treatments. The promoter of the CUT1 gene was cloned by PCR walking and 1.2 kb of the sequence 5' to the start codon was used to direct GUS expression in transformed plants. The GUS histochemical assay confirmed that the CUT1 promoter directed epidermal-specific expression in all tissues examined, in Arabidopsis as well as in a heterologous tobacco system. These results suggest that CUT1 could be important in wax production throughout the aerial parts of the plant, and that its promoter has potential as a biotechnological tool for directing epidermal-specific expression of useful genes in plants of interest.

436 Identification and Cloning of Suppressors of the Arabidopsis det1 Mutant

Jianping Hu and Joanne Chory

Plant Biology Lab and Howard Hughes Medical Institute, The Salk Institute for Biological Studies, La Jolla, CA 92037

The Arabidopsis DET1 gene encodes a nuclear protein that acts as a general repressor in multiple light-regulated signaling pathways. Dark-grown det1 plants have expanded cotyledons and short hypocotyls, and develop true leaves and chloroplasts and accumulate anthocyanins. Both dark- and light-grown det1 mutants show derepression of several light-regulated nuclear and chloroplast genes. To understand how DET1 exerts its functions in photomorphogenesis and other developmental programs, it is important to identify other components that perform in association with DET1. To this end, we took a genetic approach to screen for EMS-induced suppressors of det1. Several extragenic suppressors of det1 have been isolated in either det1-1 or det1-4 mutant background and the characterization of three of the suppressors is presented here. All three suppressors significantly suppress the dark phenotype of det1 and partially rescue the dwarf stature of det1 as adults. Our data indicate that suppression is not achieved by alterations of the size or quantity of the det1 transcript. Both ted7 and ted8 are recessive mutations that suppress the det1-4 allele. ted3, identified from a previous screen from the det1-1 background (Pepper and Chory, 1997), contains a dominant allele. In addition to suppressing the dark phenotype of det1, ted3/det1-1 appears to have longer hypocotyls than those of the wildtype while grown in white or far-red light. Positional cloning of TED3 is currently in progress. TED3 has been fine-mapped to the bottom of chromosome 1, around 130 cM on the RI map. The cloning of the TED3 gene will shed some light on the regulatory environment of DET1 and help us further understand the mechanisms by which light controls plant development via the complex network of signal transduction pathways.

437 A Pleiotropic Mutant with Altered Root Waving Phenotype on Tilted Agar Plates

Kai F. Hung, Kathleen C. Carroll, Tara H. Schulz, Patrick H. Masson

University of Wisconsin - Madison, 3302 Genetics/Biotech, 445 Henry Mall, Madison WI 53706

Using the Ac/Ds transposon system, a mutant, *wvc16*, with aberrant root waving phenotype on tilted agar surface was identified in *Arabidopsis thaliana*. Additional phenotypes were subsequently identified in this mutant. The rosette and cauline leaves of the mutant curl, whereas the siliques grow in a kinked fashion. Also, the stems curl into loops, some of which uncurl over time, and the pedicels often show a downward curvature. The mutant's root and hypocotyl show a normal response to gravity in light, but the hypocotyl exhibits a slightly abnormal phenotype in the dark assay. In auxin sensitivity assays involving IAA and 2,4-D, the mutant roots show a wild type growth response. However, the rate of root growth in the mutant seems to be more sensitive to the ethylene precursor ACC. Initial characterization indicates that the mutation is closely linked to the Ds insertion and that it is at least semi-dominant. The Ds element is inserted into the predicted first exon of a novel gene. Genetic, molecular, and physiological studies are being carried out to further elucidate the nature of the *wvc16* mutation.

438 Attenuation of phyB-signaling by SRL1 locus in *Arabidopsis*

Enamul Huq, Yurah Kang, Karen J. Halliday, Minmin Qin and Peter H. Quail
USDA/Plant Gene Expression Center/UC Berkeley, 800 Buchanan St., Albany, CA94710

As part of an effort to isolate new *Arabidopsis* mutants specifically defective in responsiveness to red light, we identified *srl1* (short hypocotyl in red light) by screening an EMS-mutagenized M2 population derived from a phytochrome B (phyB)-overexpressor line (ABO). The *srl1* mutant shows enhanced responsiveness to continuous red, but not far-red light, in both wild type and ABO backgrounds, consistent with involvement in the phyB signaling pathway, but not that of phyA. The hypersensitive phenotype of *srl1* is not due to overexpression of endogenous phyA or phyB, and the locus maps to the center of chromosome 2, distinct from any other known photomorphogenic mutants. *srl1* seedlings display enhancement of several phyB-mediated responses, including shorter hypocotyls, more expanded cotyledons, shorter petioles and modestly higher levels of CAB-gene expression under red light than the wild type. Double mutant analyses show that the hypersensitive phenotype of *srl1* is completely phyB-dependent. The data suggest, therefore, that SRL1 may encode a negatively acting component specific to the phyB-signaling pathway.

439 GI is a nuclear protein involved in phyB-signaling in *Arabidopsis*

Enamul Huq, James M. Tepperman and Peter H. Quail
USDA/Plant Gene Expression Center/UC Berkeley, 800 Buchanan St., Albany, CA94710

In a genetic screen of available T-DNA-mutagenized *Arabidopsis* populations for loci potentially involved in phytochrome (phy) signaling we identified a mutant that displayed reduced seedling deetiolation under continuous red light, but not under continuous far-red light. This behavior suggests disruption of phyB, but not phyA signaling. We have cloned the mutant locus using the T-DNA insertion and found that the disrupted gene is identical to the recently described GIGANTEA gene identified as being involved in control of flowering time. The encoded GI polypeptide has no sequence similarity to any known proteins in the database. However, using GUS-GI and GFP-GI fusion constructs, we have shown that GI is constitutively targeted to the nucleus in transient transfection assays.

Optical sectioning using the GFP-GI fusion protein showed green fluorescence throughout the nucleoplasm. Thus, contrary to previous computer-based predictions that GI would be an integral plasmamembrane-localized polypeptide, the data here indicate that it is a novel, nucleoplasmically localized protein. This result is consistent with the proposed role in phyB signaling, given recent evidence that early phytochrome signaling events are nuclear localized.

440 Transcriptional regulation of the high affinity phosphate transporters
A.S.Karthikeyan, Uthappa Mukatira, Deepa Varadarajan, M. Paino D'Urzo and K.G. Raghothama

Department of Horticulture and Landscape Architecture, Purdue university, West Lafayette-IN 47607-1165

Phosphate (Pi) is one of the least available plant nutrients in the rhizosphere. Increased expression of the high-affinity phosphate transporters is a characteristic response of plants to Pi deficiency. Although Pi transporter's expression is regulated by changes in the availability of the Pi, the mechanism of regulation is not completely understood. In order to understand the molecular events that lead to the expression of these genes, promoters of the high affinity phosphate transporters (AtPT1 and AtPT2) were fused to reporter genes firefly luciferase (LUC), β -glucuronidase (GUS) and green fluorescent protein (GFP). These chimeric clones were used to generate transgenic Arabidopsis and tobacco plants. Analysis of transgenic plants indicated that the reporter genes are expressed under the regulation of phosphate transporter promoters. The Arabidopsis promoters are capable of driving the reporter gene expression in tobacco. The expression is intimately associated with the availability of Pi in the media. The reporter genes were preferentially expressed in roots of Pi starved plants. Highest expression was observed in the regions behind root tips and root to shoot junctions. The root hairs of Pi starved plants had pronounced expression of reporter genes. During prolonged Pi starvation, expression of reporter genes was also noticed in senescing leaves and inflorescence. These results suggest that the Pi transporter (AtPT2) is not only involved in Pi acquisition but may also play a role in redistribution of the nutrient during prolonged starvation. Studies are in progress to further characterize the transcriptional regulation of phosphate transporters.

441 Characterization of an Arabidopsis early-phytochrome-responsive gene encoding a novel MYB-related protein initially identified by a fluorescent differential display screening

Norihito Kuno¹, Simon Geir Møller², Tomoko Shinomura¹, Nam-Hai Chua² and Masaki Furuya¹

¹Hitachi Advanced Research Laboratory, Saitama 350-0395, JAPAN, ²The Rockefeller Univ., New York, NY 10021, USA

Light-regulated expression of various nuclear genes is mediated by phytochromes. To elucidate the molecular mechanism of phytochrome-regulated gene expression, early-phytochrome-responsive genes are useful molecular probes since they are directly controlled by the phytochrome signal transduction chain. In this study, we used a fluorescent differential display (FDD) 1 technique to isolate Arabidopsis early-phytochrome-responsive genes. Wild type six-day-old etiolated seedlings were irradiated with a pulse of 1 mmol m⁻² red light and then returned to darkness. Total RNA was

extracted from these seedlings after 0, 30 and 60 min light treatment. mRNA fingerprints of these samples were compared in order to identify target genes. From ca. 20,000 displayed bands, five differentially expressed bands were identified. Sequence analysis revealed that the five cDNAs represent three distinct mRNA species. One inducible cDNA showed 70% similarity to a MYB DNA-binding domain of the Arabidopsis Circadian-Clock Associated 1 gene2 . Using this FDD fragment as a probe, a full-length myb -related cDNA clone (1.6 kbp) was isolated from an Arabidopsis cDNA library. The isolated clone encodes a novel MYB-related protein of 346 amino acids harboring a single N-terminal MYB-domain. Transient expression experiments in onion epidermal cells, using a full-length MYB-GFP fusion protein, revealed exclusive nuclear localization of MYB-related protein. Subsequent expression analysis demonstrated that both PhyA and PhyB could induce expression of this myb -related gene and moreover that the phytochrome-mediated induction was not blocked by protein synthesis inhibitor. To probe the functionality of the isolated gene product, we transformed Arabidopsis with an antisense construct harboring the FDD fragment. The antisense transgenic plants grown under continuous red light showed physiological and molecular phenotypes suggesting a function for this myb -related gene in the phytochrome-signal transduction pathway.

1Kuno et al. (2000) Plant Physiol. 122 :15-24. 2Wang et al. (1997) Plant Cell 9 :491-507.

442 Molecular Approaches to Characterize High Affinity Nitrate Transport in Arabidopsis thaliana

Georg Leggewie, Anja Günther, Thomas Keller, Katrin Piepenburg and Michael Udvardi
Max-Planck-Institute for Molecular Plant Physiology, Postfach, D-14424 Potsdam, Germany, email: leggewie@mpimp-golm.mpg.de

In most of our aerobic agricultural soils nitrate is the main source of inorganic nitrogen. Plants possess at least three different nitrate uptake systems, one low affinity system (LATS) and two high affinity systems (HATS). The latter can be subdivided in a constitutive (cHATS) and an inducible (iHATS) component. Four different genes are known in Arabidopsis that may be part of the iHATS (AtNRT2.1 to AtNRT2.4). These NRT2 genes belong to the nitrate-nitrite porter (NNP) family of membrane transporter genes which is one out of seventeen families that form the Major Facilitator Superfamily (MFS) (Forde and Clarkson, 1999). Members of this family share a similar topology where two transmembrane domains, each spanning the membrane six times, are separated by one hydrophilic loop.

We are presently studying the transcriptional expression pattern of the different NRT2 genes. AtNRT2.1 is known to be induced by nitrate following a period of nitrogen starvation. Ammonium suppresses AtNRT2.1 transcription. However, the transcriptional expression of these genes in response to other nutritional or environmental stresses has not been studied in detail. We are also looking into the possibility that the NRT2 transporters are regulated by phosphorylation. Total RNA has been isolated from roots of Arabidopsis plants grown under different N-regimes. First strand cDNA was used to screen an EST-microarray. This microarray contains cDNAs encoding known Arabidopsis phosphatases and kinases. Ten clones are presently under investigation that show differential expression. The transcription of genes encoding these ESTs is being

tested using real time PCR. This approach may also identify putative regulatory genes which are involved in other physiological reactions in response to varying nitrate regimes. Results will be discussed in respect to their significance for high affinity nitrate uptake into the plant.

Forde, B.G. and Clarkson D.T. (1999) Nitrate and ammonium nutrition of plants: Physiological and molecular perspectives. *Advances in botanical research incorporating advances in plant pathology*, 30 : 1-90.

443 Ancient classes of metallothionein-like genes in *Arabidopsis thaliana*

Anne Marie and Richard B. Meagher

University of Georgia, Life Sciences Building, Athens GA 30602-7223

Plants maintain a delicate balance of essential heavy metals, such as copper, zinc and nickel at micronutrient levels while excluding toxic metals such as cadmium, arsenic and mercury. Their mechanisms for regulating metal concentrations are largely unknown though a few important classes of proteins have been identified. Plant metallothionein-like proteins (MTs) are a class of proteins that are suspected to be mechanistically important for the regulation of these optimal metal concentrations. Plant MTs are low molecular weight proteins, typically between 45-100 amino acids, with cysteine rich metal coordination domains (Cys-Xn(1-6)-Cys) linked by a region containing variable amino acids. The cysteine residues in each domain are suspected to coordinate metal ions, as opposed to forming covalent bonds, based on data from the crystallographic structure for rat liver MT. Sequence alignments and protein trees of over 50 plant sequences have been made using programs available through GCG (PAUP, pileup, neighbor joining etc.). To date, we have strong evidence for the existence of four ancient classes MTs in *Arabidopsis* based on protein sequences and cysteine spacing patterns. Several other isolated plant MT sequences suggest the final number may be closer to six or eight. Representatives from the ancient classes of *Arabidopsis* MTs have been cloned from cDNA libraries. These clones will be used to complement yeast strains knocked out for the endogenous two yeast MTs. We are interested in why these ancient classes have been conserved. Toward this end, we will attempt to determine the metal specificity, differential regulation and subcellular localization of various MTs.

444 DET1 Responsive Elements on the CAB2 Promoter

Bridey B. Maxwell, Daniel Poole and Joanne Chory

The Salk Institute for Biological Studies, 10010 North Torrey Pines Rd., La Jolla CA 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. Light grown *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. Both the timing and location of expression of the light-regulated CAB2 gene is aberrant in *det1*. 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 compared their expression in wildtype vs. *det1*

seedlings. We have discovered that the DET1 responsive element (DtRE) responsible for CAB2::LUC overexpression in dark-grown det1 seedlings 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 action. In the light, the underexpression of the CAB2 promoter in the det1 mutant requires the CUF-1 element but not sequences containing the DtRE. In roots, DET1 mediates repression of CAB2 expression via both the CUF-1 element and DtRE-containing sequences. 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 requires the DtRE for activation in roots but not in cotyledons. In addition, we have discovered that there are at least two factors present in Arabidopsis extracts which specifically bind the DtRE and that the binding of these factors is altered in det1 mutant extracts: a potential activation factor which binds the sequence CAAAACGC and a potential myb-like factor which binds AAAAATCA.

445 Regulation and Functions of Calmodulin-Related TCH2 and TCH3

E. McCormack, K. A. Johnson, A. C. Hughes, P. Campbell, and J. Braam
Rice University, 6100 Main St, Houston, TX 77005

Calmodulin is a major calcium receptor in eukaryotic cells and functions to mediate responses to changes in levels of calcium acting as a second messenger. We have identified two calmodulin-related genes, called TCH2 and TCH3, in Arabidopsis that show strong upregulation of expression in response to a variety of environmental stimuli, including touch, darkness and temperature shocks. TCH::GUS expression and TCH3 protein accumulation correlate with sites of mechanical strain and cell expansion; these data indicate that the TCH2 and TCH3 functions may be required at these sites during plant growth and development and may be additionally necessary during responses to environmental stress.

TCH2 and TCH3 share 44% and 60% amino acid sequence identity with calmodulin and can bind calcium; it is likely, therefore, that the TCH proteins function to mediate responses to calcium fluctuations. Sequence divergence from calmodulin, however, indicates that the targets of TCH2 and TCH3 are likely to be distinct from those of calmodulin.

We are searching for proteins that interact with TCH2 and TCH3 in a calcium-dependent manner to identify potential targets. The three dimensional structure of TCH2 is being investigated. In addition, we are identifying plants with altered expression of and mutations in the TCH2 and TCH3 genes to elucidate the physiological functions of the proteins.

446 Study of plastid signal transduction: GUN5 encodes for a H-subunit of Mg-chelatase

Nobuyoshi Mochizuki(1), Judy Brusslan(2), Robert Larkin(3), Akira Nagatani(1), Joanne Chory(3)

(1) Kyoto University, (2) California State University, (3) The Salk Institute

Plastid-derived signal, so called "plastid signal", plays an important role for the coordinated expression of nuclear genes encoding for photosynthesis-related proteins with the functional state of chloroplast in plants. For instance, transcription of CAB genes are strongly repressed when plants are treated with Norflurazon (a potent inhibitor of

carotenoid biosynthesis, which leads to an arrest of chloroplasts at the early stage of development under a strong light.) We have been studying Arabidopsis gun (genome uncoupled) mutants that have CAB expression in the presence of Norflurazon. There are five GUN loci identified; GUN1-GUN5. In gun mutants, CAB expression is altered only in the aspect of plastid-dependent regulation, but not affected in the other aspects such as light- and organ-specific regulation. On the other hand, there is obvious reduction of chlorophyll accumulation in gun4 and gun5 mutants. gun5 and cch (a paler allele of gun5) have reduced level of Mg-ProtoIX production, suggesting that they have a defect in Mg-chelatase activity. By a positional cloning, we found that GUN5/CCH encodes for a largest subunit (ChlH) of Mg-chelatase (comprised of ChlH, ChlD, ChlI). We examined a T-DNA insertion mutant allele (cs) of chlI for gun phenotype, and found that the cs mutant failed to show CAB de-repressed phenotype (i.e. gun phenotype) in the presence of Norflurazon. The Mg-chelatase activity appeared to be lower in cs mutant than in gun5 according to an indirect enzyme assay. Therefore, we hypothesize that the Mg-chelatase H-subunits has distinctive functions, one as a subunit of the enzyme, and the other as a mediator of plastid signal. We are currently testing for a gun-like phenotype in a series of barley xantha mutants that are known to have defects in Mg-chelatase subunits. We are also searching T-DNA tagged lines for a chlD mutant to understand the different involvement of Mg-chelatase subunits on the plastid signaling. Possible involvement of chlorophyll biosynthesis intermediates in the plastid signaling will be also discussed.

447 NPH3 is a novel putative phosphoprotein that interacts with NPH1, a photoreceptor mediating phototropism in Arabidopsis

Andrei Motchoulski and Emmanuel Liscum

Department of Biological Sciences, 105 Tucker Hall, University of Missouri-Columbia, Columbia MO 65211

Previous physiological and photobiological studies of nph3 mutants showed that that NPH3 is specifically involved in phototropism, acting genetically close to NPH1. To reveal the molecular mechanisms of blue light-dependent signaling we have cloned the NPH3 gene via positional cloning. The predicted NPH3 protein is a novel protein with no obvious homologies to proteins of known enzymatic functions. However, searches of the Arabidopsis database identified >30 predicted proteins that are highly homologous to NPH3. Cellular partitioning experiments revealed that NPH3 is plasmalemma associated, and its membrane localization does not change in response to blue-light irradiation. However, NPH3 from blue, but not red, light-irradiated seedlings exhibited enhanced mobility on SDS-PAGE, which could be blocked by in vivo application of the protein phosphatase inhibitor, okadaic acid, or the protein kinase C inhibitor, staurosporin. These results suggest that NPH3 is a phosphorylated protein in the dark and gets dephosphorylated in response to blue light. Given the genetic coupling of NPH1 and NPH3 and co-localization of their encoded proteins to the plasmalemma, we used yeast two-hybrid and in vitro co-immuno precipitation approaches to examine whether these proteins might directly interact. We found that, C-terminal portions of NPH3 that include the coiled-coil domain strongly interacted with the N-terminal two-thirds of NPH1, which includes the chromophore-binding region but not the kinase domain. We hypothesize that NPH3 acts as a scaffold/adaptor protein to bring together/activate components of a

NPH1-dependent multimolecular phosphorelay that functions in early phototropic signaling.

448 The kinetics of Arabidopsis seedling growth as a tool for studying photomorphogenesis

Brian M. Parks, Kevin M. Folta, Ryan J. Magana, and Edgar P. Spalding
Department of Botany, University of Wisconsin, Madison, WI 53706

Light is one of the most important environmental factors affecting plant growth and development, incorporating the action of multiple phytochromes and blue-light photoreceptors. In recent years, it has become increasingly clear that these photoreceptors possess both distinct and overlapping roles in photomorphogenesis. In this regard, the analysis of photomorphogenic mutants has been indispensable in attributing specific functional roles to the action of particular photoreceptors. However, kinetic information that could be used to determine when particular photoreceptors and their downstream transduction elements act has been lacking in general. High-resolution techniques for measuring hypocotyl growth in cry1 mutant seedlings of Arabidopsis have enabled us to determine when cryptochrome1 exerts its effect in blue light (*Plant Physiol*, 118: 609-615). We are now extending this work to include a cry2 mutant and a cry1 cry2 double mutant in order to complete the genetic dissection of the response kinetics involving this blue-light photoreceptor family. For red-light-controlled growth, we have found that phytochromes A and B are temporally coordinated so as to regulate different phases of hypocotyl growth (*PNAS*, 96: 14142-14146). We have also begun to analyze the red-light-induced growth response kinetics of spa1, a suppressor mutant of phyA. In a wild-type background, spa1 seedlings have shorter than normal hypocotyls in both red and far-red light, and it has been shown that SPA1 acts as a phyA-induced positive regulator of growth (Hoecker et al, *Plant Cell*, 10: 19-33). We will present our analysis of growth kinetics for spa1-3 seedlings in response to red light and show that SPA1 influences growth during a period overlapping and extending beyond the window over which phyA predominately affects growth rate. This type of analysis will help better define the role of this element in the scheme of complex developmental changes that are initiated upon a seedling's emergence into an illuminated environment.

Supported by NSF grant IBN-9974585, and USDA grant 99-01833.

449 Analysis of function of NPH1 homolog, NPL1

Tatsuya Sakai (1), Toshitaka Kagawa (2), Masahiro Kasahara (3), Trevor Swartz (3), Noriyuki Suetsugu (4) Kazusato Oikawa (4), Sumie Ishiguro (1), Tomohiko Kato (5), Satoshi Tabata (5), Winslow Briggs (3), Masamitsu Wada (4), Kiyotaka Okada (1)
(1) Dep. Bot., Grad. Sch. Sci., Kyoto Univ., Japan. (2) Natio. Ins. Basic Biol., Japan. (3) Dep. Plant Biol., Carnegie Institution of Washington, USA. (4) Dep. Biol., Fac. Sci., Tokyo Metropolitan Univ. Japan. (5) Kazusa DNA Research Institute, Japan

The blue light receptor NPH1 (for nonphototropic hypocotyl) has been considered to be the only UV-A/blue light receptor that induces a phototropic response by the hypocotyl and root of Arabidopsis. However, we showed the involvement of another blue light receptor in phototropism at high fluence rate of blue light ($-100 \mu\text{mol}/\text{m}^2/\text{sec}$). To identify this second receptor, we made double mutants of the nph1 mutant with other blue

light receptors, cry1, cry2. *nph1cry1* and *nph1cry2* mutants also showed the hypocotyl phototropism at high fluence rate of blue light, and they seemed not to be the second receptor.

Jarillo et al. (1998) reported the isolation of an NPH1-related gene, NPL1. Like NPH1, NPL1 also possesses LOV domains and a kinase domain like NPH1. We hypothesized that NPL1 is the second receptor, and isolated a T-DNA-tagged line at the NPL1 locus from the pool of T-DNA-tagged lines at Kazusa DNA Research Institute. T-DNA was inserted in the 8th exon and we confirmed that NPL1 is null in this mutant by the northern blot analysis. The expression pattern of NPL1 mRNA showed that NPL1 expresses in all tissues without root and was induced by blue light slightly.

Now, we found the phenotype of the *np11* mutant, though it is not involved in the phototropism. We will present function of the NPL1 protein as a novel blue light receptor in Arabidopsis.

450 DET1 Structure/Function Analysis

Dana F. Schroeder, Manfred Gahrtz, R. Kim Cook, and Joanne Chory
Howard Hughes Medical Institute and Plant Biology Lab, The Salk Institute, La Jolla, CA 92037

Light exerts a powerful influence on plant development. A particularly dramatic example of this occurs during seedling development. Arabidopsis seedlings grown in the light exhibit short hypocotyls, open cotyledons, and chloroplast development while seedlings grown in the dark exhibit an etiolated phenotype, consisting of long hypocotyls, closed cotyledons forming an apical hook, and lack of chloroplast development. Genetic screens in several labs have identified mutants that exhibit a light grown, or de-etiolated, phenotype even in the dark. The first of these mutants identified was *det1*. *det1* has been cloned and found to encode a 62kD novel nuclear protein. Using an N-terminal 6xmyc epitope tag, DET1 is detected primarily in an ~350kD complex in extracts of both light- and dark-grown seedlings, as well as in a variety of mutant backgrounds. This construct fully rescues the *det1* seedling phenotype. An N-terminal GFP-tagged DET1 also results in full rescue and formation of the 350kD complex, and is nuclear localized. However a C-terminal GFP-tagged DET1 results in only partial rescue and does not exhibit significant nuclear localization. The 350kD complex is not detected in extracts of these lines, the majority of protein instead being detected in a ~550kD complex (a minor component in extracts of the N-terminally-tagged lines). These results suggest a correlation between the 350kD complex, nuclear localization and DET1 function, and a requirement for the DET1 C-terminus in these processes. C-terminal truncations and mutations are being introduced into the N-terminal GFP-tagged DET1 to further define these interactions. In order to identify the other components of the DET1 complex, 6xmyc-DET1 was introduced into tobacco BY2 cells. The 350kD complex is also detected in extracts of these transformed cells, while DET1 produced in a baculovirus system forms only monomers. These results suggest that the transformed BY2 cells will be an appropriate source of the DET1 complex for purification studies.

451 SKU5 encodes a putative GPI anchored, cell wall modifying enzyme essential for proper root growth and environmental responsiveness

John C. Sedbrook, Kathleen L. Carroll, Kai F. Hung, Patrick H. Masson, and Christopher R. Somerville

Carnegie Institution, Department of Plant Biology, 260 Panama St., Stanford CA 94305
Since plants are immobile, they need to direct the growth of their organs in order to become optimally positioned to acquire the essential elements within their surrounding environment. To learn more about the cellular expansion and/or cell division processes underlying directed root tip growth, we employed a root waving assay to identify a T-DNA tagged mutant named *sku5*. Root waving is a process where roots form a sinusoidal wave pattern as they grow down a tilted agar surface. Root waving is likely caused by root tip responses to gravity and touch, coupled with a circumnutation-like process. The roots of *sku5* plants skew from the normal vertical direction of waving to a horizontal one. *sku5* roots also grow to be slightly shorter than wild type. Kinetic analysis of the gravity response of *sku5* roots embedded in an agar medium showed a decrease in the rate of root tip bending when compared to wild type. *SKU5* encodes a putative 587 amino acid polypeptide with 20 to 25 percent sequence identity to ascorbate oxidases and laccases- two protein families of so-called blue copper oxidases. *SKU5* appears to not be a blue copper oxidase because it lacks the highly conserved motifs necessary for copper binding. Computer modeling programs predict the *SKU5* protein to contain hydrophobic domains at each end. This type of domain arrangement is typical of proteins which get glycosyl-phosphatidylinositol (GPI) modified. GFP and GUS reporter constructs along with Western blot analysis using an anti-*SKU5* antibody show that *SKU5* is expressed in all organs. *SKU5* cellular localization and enzymatic activity will be discussed.

452 Characterization and cloning of *ZEITLUPE*, a novel component of the plant circadian clock system

David E. Somers*¹, Thomas F. Schultz*, Maureen Milnamow² and Steve A Kay*

*The Scripps Research Institute, Division of Plant Biology/Department of Cell Biology, ¹Department of Plant Biology, Ohio State University, ²Novartis Agricultural Discovery Institute

We have characterized and cloned a locus (*ZEITLUPE*; *ZTL*) responsible for mediating the light-dependent control of the circadian period in *Arabidopsis*. The *ZTL* locus is defined genetically by two mutations (*ztl-1*, *-2*), which have very similar long period phenotypes as measured by a *CAB2::luciferase* luminescence assay. Fluence-rate response tests show a heightened fluence-rate effect of red and blue light on period length in these mutants. At high light intensities ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$) period is 2-3 h longer than wild type (WT), whereas at low fluence rates ($1-2 \mu\text{mol m}^{-2} \text{s}^{-1}$) *ztl* mutations increase period by 9-10 h. Further tests with *ztl-1* show that the rhythms of cotyledon and leaf movements are also lengthened, indicating that two very different clock-controlled processes, transcription and cell expansion, are affected by this mutation. Flowering time in long days is increased, relative to WT, also consistent with a defect in regulation by the circadian clock.

In contrast, in *ztl-1* the acute (rapid) response of *CAB2* gene expression to a red or blue light pulse is identical to WT and there is only a slight effect of light intensity on hypocotyl length, suggesting that *ZTL* may be primarily dedicated to controlling light input to the circadian clock. In both the WT and *ztl-1*, *ZTL* message levels remain constant under light/dark cycles and in continuous light, indicating that *ZTL* gene

expression is neither light regulated nor under circadian clock control. ZTL is part of a three-member gene family and encodes a novel 609 amino acid polypeptide consisting of an amino-terminal PAS-like LOV domain, an F-box and six carboxy-terminal kelch repeats. This unique domain composition suggests that ZTL may play a role at the interface between light input to the clock and the oscillator itself.

453 Functional Dissection of Arabidopsis Transcriptional Activator CBF1

Stockinger EJ1, Zarka DG2 and MF Thomashow2

1The Ohio State University, 2Michigan State University

To survive extremes in low temperature many temperate plants including *Arabidopsis thaliana*, increase their threshold of freezing tolerance through cold-acclimation. This increase in freezing tolerance is brought about by low, nonfreezing temperatures prior to an otherwise killing-temperature. A key initial event, observable within minutes of transferring *Arabidopsis* plants to a low temperature environment, is the rapid accumulation of mRNAs encoded by the CBF (C-repeat binding factor) genes (1). The CBFs then induce transcription of the COR genes by binding to the CRT/DRE (C-repeat/dehydration-responsive element; 2), a cis-acting DNA regulatory element present in the COR gene promoters (3,4) and activating COR gene transcription (1,2,5). In turn the COR gene mRNAs encode protein products that function in a structural role to increase the freezing tolerance of the plant (6). A current objective is to identify regions of CBF1 essential for activity. Towards this end we created chimeric activator proteins in which different domains of CBF1 are fused to domains from other transcriptional activators. The activity of these chimeric constructs was then assayed in yeast, in *Arabidopsis* transient assays and in transgenic *Arabidopsis* plants. In transient assays, full-length CBF1 activated transcription from the native COR15a promoter and the synthetic CRT/DRE CaMV35S(-46) minimal promoter. Fusion of the N-terminal 115 amino acids of CBF1, which includes the AP2 type DNA binding domain, to the activation domain of the Herpes Simplex Virus activator protein VP16, also sufficed to direct CBF1 to the COR15a promoter. When the C-terminal 98 amino acids of CBF1 were fused to the DNA binding domain of the yeast GAL4 protein, the recombinant protein activated transcription both in yeast and in *Arabidopsis*. This research was supported in part by grants (to M.F.T.) from the USDA (NRICGP), the National Science Foundation and the Michigan Agricultural Experiment Station.

(1) Gilmour et al. (1998) *Plant J.* 16:433-442.

(2) Stockinger et al. (1997) *Proc. Natl. Acad. Sci. USA* 94:1035-1040.

(3) Baker et al. (1994) *Plant Mol. Biol.* 24:701-713.

(4) Yamaguchi-Shinozaki and Shinozaki (1994) *Plant Cell* 6:251-264.

(5) Jaglo-Ottosen et al. (1998) *Science* 280:104-106.

(6) Artus et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:13404-13409.

454 Isolation and characterization of mutants in the phytochrome-mediated enhancement of phototropism

Emily L. Stowe-Evans, Emily Bradford, Emmanuel Liscum

University of Missouri-Columbia

Plants adapt to changes in their light environment by altering their growth and development. Etiolated *Arabidopsis* seedlings respond to directional blue light cues through a differential growth process called phototropism. Mutational studies in *Arabidopsis* have demonstrated that a minimum of five loci are involved in blue light-induced seedling phototropism: NPH1 (NONPHOTOTROPIC HYPOCOTYL 1), NPH2, NPH3, NPH4, and RPT2 (ROOT PHOTOTROPISM 2). Interestingly, *nph4* null mutants, while exhibiting no phototropic response as etiolated seedlings exposed to low fluence rate blue light, show a respectable phototropic response under conditions where both phototropin (the blue light receptor encoded by the NPH1 locus) and phytochromes are activated (i.e., unilateral UV-A or red plus unilateral blue light). This conditional phenotype has allowed us to genetically separate the basal blue light-induced phototropic response from the phytochrome-dependent enhancement of phototropism that is a component of the adaptation response. In an effort to understand the molecular bases for the interaction of the phototropin and phytochrome-dependent pathways, we have selected second-site mutants in the *nph4* background that failed to exhibit the phytochrome-dependent recovery of phototropic responsiveness. Physiological and genetic characterization of four such "*nph4*-enhancer mutants", as well as analyses of single and multiple phytochrome mutants will be presented.

This work was supported by a grant to E.L. from NSF (MCB-9723124). E.L.S.-E. was supported by a predoctoral fellowship from the University of Missouri Maize Biology Training Program, a unit of the DOE/NSF/USDA Collaborative Research in Plant Biology Program.

455 Signaling function of tobacco PP2C (NtPP2C1) and its *Arabidopsis* homologue (AtPP2CA) in stress responses

Eva Vranová¹, Christian Langebartels², Rutchadaporn Sriprang¹, Sari Tähtiharju³, Hilde Willekens¹, Dirk Inzé¹, Wim Van Camp¹

¹Dept. Plantengenetica, VIB-Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium; ²GSF-Forschungsz. für Umwelt und Gesundheit, D-85764

Neuherberg, Germany; ³Dept. Biosc., Fin-00014 University of Helsinki, Finland

A protein phosphatase 2C (PP2C)-homologous cDNA (NtPP2C1) was isolated from *N. tabacum* in a differential screen for genes implicated in adaptive responses to oxidative stress. The NtPP2C1 deduced protein sequence is most similar (69%) to the AtPP2CA, an *Arabidopsis* PP2C implicated in abscisic acid (ABA) signaling, and possesses also GxxDGHG conserved motif that is characteristic for this class of PP2Cs. Sequence data thus suggest that tobacco NtPP2C1 is a functional homologue of PP2Cs implicated in ABA signal transduction. NtPP2C1 is strongly and persistently induced by drought stress, which further support this assumption. We have observed that in contrast to its transcriptional upregulation by drought stress, NtPP2C1 is downregulated by oxidative stress and heat shock. If NtPP2C1 functions as a negative regulator in ABA signaling like its homologues in *Arabidopsis*, downregulation of NtPP2C1 expression by oxidative stress or heat shock can sensitize the plant to ABA. The result suggest that NtPP2C1 may constitute a point of convergence between heat shock, oxidative stress, and drought/ABA signaling pathways. To confirm our hypothesis and to study the signaling function of this phosphatase in great detail, we have recently focused on the AtPP2CA, a putative

Arabidopsis orthologue of NtPP2C1. Some preliminary data from the study on AtPP2CA will be presented in addition to above described data on tobacco NtPP2C1.

456 Translocation of a large DNA fragment from chromosome 3 into *Glabra2* promoter on chromosome 1 caused glabrous, high pigment, and short hypocotyl phenotypes in *Arabidopsis pig2* mutant
Lihua Wu 1,2, Atsushi Tanaka 3, Shigemitsu Tano 3, Kim Findlay 4, Cathie Martin 4, Jeremy Harbinson 5, Yasunori Nakamura 1,6, Masashi Ugaki 1,7, Hiroaki Ichikawa 1
1 Natl. Inst. Agrobiological Resources, Jpn, 2 Bio-Oriented Technology Research Advancement Institution, Jpn, 3 Plant Resources Lab., JAERI, Jpn, 4 Agrotechnological Res. Inst., NL, 5 John Innes Centre, UK, 6 Akita Pref. Univ., Jpn, 7 Univ. Tokyo, Jpn
To uncover the regulatory mechanism of anthocyanin accumulation in response to light irradiation, an *Arabidopsis* mutant with elevated level of anthocyanin pigments was isolated among M2 seedlings with ion-beam irradiation. Mutant seedlings grown under continuous yellow light accumulated a few-fold more pigments and showed inhibition of hypocotyls elongation than its equivalent wild type. The mutant line also had glabrousness and increased root hairs. The glabrous phenotype was recessive and allelic to *glabra2* (*gl2*) mutation on chromosome 1. Accordingly, the mutant line was designated "pig2" for pigment intensified and *glabra2*. *pig2* plants also showed elevated chlorosis with decreased activity of photosynthesis when grown in a medium containing 2% sucrose under continuous white light. DNA gel blot analysis indicated that rearrangement(s) occurred around initiation codon in the *GL2* gene of *pig2* mutant. Analysis of 5'-upstream sequence of the *GL2*-coding region in *pig2* by PCR-based genome walking method revealed that a 41-kbp fragment derived from chr. 3 was inserted at approx. 50 bp 5'-upstream from exon1 of the *GL2* gene. Analysis of *GL2* transcripts by RT-PCR/Southern strategy detected reduced amounts of larger transcripts only in *pig2*. Sequencing of the *GL2* cDNA from *pig2* indicated immature splicing of the transcripts. These data suggested that a translocation of the large DNA fragment from chr. 3 to chr. 1 caused physical separation between *GL2*-coding and *GL2* promoter region. On the contrary, a deletion of the 41-kbp fragment on chr. 3 was detected in *pig2* genomic DNA. In progress is molecular genetic dissection of *pig2* genome to elucidate one-to-one correspondence between each phenotype and genotype/locus/gene.

457 Light-regulated signal transduction:the phytochrome signaling cascade in *Arabidopsis*
Lu-Shu Yeh, Meng Chen, Tedd D. Elich, Yelena Belkin and Joanne Chory
Plant Biology Laboratory, Howard Hughes Medical Institute, Salk Institute, 10010 N. Torrey Pines Rd., La Jolla CA92037
Plants have a battery of photoreceptors to monitor ambient light conditions. They use these photoreceptors to detect the quality, quantity and duration of light, and to determine if they are being shaded by other plants. Previous studies revealed several classes of which the photoreceptors in plants and red/far-red receptors are known as phytochromes. Although phytochromes were discovered long ago, limited information is known about the phytochrome signaling pathway. By using a yeast interaction-trap system, our lab has isolated two genes that act in the pathway(s): PAB1 (phytochrome and actin binding protein) and PRP1 (phytochrome related phosphatase). PAB1 contains four kelch repeats.

Kelch-containing proteins are known to interact with actin. PAB1 binds actin in a Ca²⁺-dependent way in vitro. PAB1 overexpressors have a longer hypocotyl in far-red light in a PHYA-dependent manner. The adult *pab1* mutant (from Dr. Joe Ecker's collection) has smaller rosette and cauline leaves. A CFP-PAB1 line shows a web-like pattern of localization in the cytoplasm. This data suggest that PAB1 is associated with cytoskeleton in vivo. Thus, PAB1 may play a role in phytochrome-regulated cell expansion. PRP1 is ~30% identical to protein phosphatase 2C, a known class of negative regulators of signaling pathways. Recombinant PRP1 protein has serine/threonine phosphatase activity in vitro. Overexpression of the PRP1 catalytic domain results in plants that are less sensitive to light. The PRP1 knockout (from Joe Ecker's collection) is more sensitive to white light (50 μmoles). These data suggest that PRP1 is involved in negatively regulating the phytochrome signaling pathway, possibly by dephosphorylating phytochrome(s) directly. Detailed genetic and biochemical studies are underway to further elucidate the role of PRP1 in phytochrome signaling. Thus, we have discovered two new genes that appear to play a role in phytochrome signaling. In the future, we will need to study all of these PHY-interacting proteins biochemically and genetically.

458 Localisation of Putative Plasmodesmata-Associated Proteins

Leila M Blackman, Bruce R Lyon, and Robyn L Overall

School of Biological Sciences, University of Sydney, Sydney, Australia

Plasmodesmata are the minute channels that allow the movement of small molecules between adjacent cells. Recent research has shown that plasmodesmata can allow the movement of specific molecules, such as transcription factors and viral movement protein/nucleic acid complexes, which are considerably larger than the size of molecules that routinely move from cell-to-cell (Mezitt and Lucas, 1996). Little is known about the molecular architecture of plasmodesmata, the regulation of their permeability or the molecular interactions that allow the movement of large molecules. We have previously identified 4 putative plasmodesmata-associated proteins from the giant fresh water alga, *Chara corallina* (Blackman et al., 1998). A monoclonal antibody against one of these, a 45 kDa protein, has been localised to plasmodesmata (Blackman et al., 1998). This antibody was used to screen a *C. corallina* expression library. Positive clones were placed in an expression system allowing the production of fusion proteins carrying the peptide marker, S.Tag, thus allowing protein detection. Tagged clones were transiently expressed in *Arabidopsis thaliana* and *Impatiens wallerana* using microprojectile bombardment and the location of the fusion proteins determined with antibodies against the S.Tag.

Blackman, L M, Gunning, B E S and Overall, R L (1998) A 45 kDa protein isolated from the nodal walls of *Chara corallina* is localised to plasmodesmata. *Plant J.* 15:401-411
Mezitt, L A and Lucas W J (1996) Plasmodesmal cell-to-cell transport of proteins and nucleic acids. *Plant Mol. Biol.* 32: 251-273

459 Plasmodesmata exhibit two modes of intercellular movement of macromolecules, targeted and non-targeted, according to parameters imposed by subcellular localization, developmental state, and environment

Katrina Crawford and Patricia Zambryski

Department of Plant and Microbial Biology, Koshland Hall, University of California, Berkeley, CA 94720

A quantitative assay for cell-to-cell communication in plants reveals that a striking number of cells transport large proteins through plasmodesmata. The leaf is exposed as a heterogeneous mix of cells with varying degrees of symplastic continuity, negating the view that only targeted proteins traffic plasmodesmata. Plasmodesmata are intrinsically dynamic with conformations fluctuating between closed, open and dilated. Superimposed upon this innate variation is the transport of targeted proteins (exemplified by viral movement proteins) that localize and traffic via plasmodesmata and facilitate the movement in trans of macromolecules. Non-targeted protein movement occurs by diffusion according to the aperture of plasmodesmata. Such diffusion fluctuates temporally and is restricted by protein size. Growth conditions and stage of leaf development dramatically affect the population size of cells trafficking macromolecules. A primary determinant of non-targeted transport is subcellular localization, as cytoskeletal-anchored and endoplasmic reticulum-sequestered GFP fusions do not move between cells. In contrast, nuclear-localized and cytoplasmic fusions freely diffuse. These results document two types of protein transport, targeted, and non-targeted, which may direct non-cell-autonomous development

460 PIP1 and PIP2 subfamilies may constitute functionally different plasma membrane aquaporin groups

Franck, K.I.1, Frangne, N.2, Affenzeller, M.1, Schaeffner, A.R.1

1GSF Research Center for Environment and Health, Institute of Biochemical Plant Pathology, D-85764 Neuherberg, Germany; 2 Laboratoire de Physiologie Végétale, Université de Neuchâtel, CH-2007 Neuchâtel, Switzerland

Major intrinsic proteins (MIP) are encoded by large gene families in mammals and plants. Most of those tested exhibit water permeability or aquaporin activity driven by a difference in water potential across the membrane. Currently 31 Arabidopsis MIP genes are known which can be grouped into 13 PIP (plasma membrane intrinsic proteins), 11 TIP (tonoplast intrinsic proteins), and 7 NLM (nodulin-like MIP, membrane localization not yet known). Based on their amino acid sequence the highly homologous PIP can be subdivided into two distinct families, five PIP1 and eight PIP2 proteins. This distinction is observed in other plant species as well. We compared the spatial and temporal expression pattern of PIP1 and PIP2 members in Arabidopsis leaves. PIP1 members show a ubiquitous expression on transcriptional and protein level that slightly decreases with leaf development. In contrast, the three most abundant PIP2 members are all confined to the region of vascular bundles and PIP2 protein levels are somewhat higher in adult leaves than in younger ones. These differences suggest that the highly homologous, yet evolutionary conserved distinct subfamilies may have different physiological roles although both enhance membrane water permeabilities. PIP1 are primarily involved in water uptake during growth whereas PIP2 serve central roles in releasing water from the vascular bundles into surrounding tissues and assist water uptake during loading of photosynthates.

461 A non-cell-autonomous function of Arabidopsis TERMINAL FLOWER 1 is exerted by developmentally regulated protein trafficking

Koji Goto

Research Institute for Biological Research (RIBS), Kayo-cho, Jobo, Okayama, 716-1241, Japan

The above ground part of plants is produced from the shoot apical meristem (SAM), which consists of three distinct cell layers, called L1, L2, and L3. Most cell divisions within each layer produce cells of the same layer. Therefore, interlayer cell-cell communication plays a critical role in coordinate cell proliferation and differentiation during the formation of leaf and flower primordia. The TERMINAL FLOWER 1 (TFL1) gene of Arabidopsis serves a key function in the differentiation of SAM during development especially in the establishment and maintenance of inflorescence meristem (IM). However, TFL1 is expressed only in the subapical region (inner region of L3 layer) of the vegetative and inflorescence meristem. This observation suggests that TFL1 acts non-cell-autonomously and that the function of TFL1 may involve intercellular signalling. To reveal the molecular mechanism of the non-cell-autonomous function of TFL1, we observed the localization of TFL1 protein using fusions of green fluorescent protein (GFP) and TFL1. In this study, we demonstrate that the TFL1 protein is transmitted from L3 to the outer layers, and suggest that this protein trafficking is regulated by the developmental phase of the SAM. We also indicate that the ectopic L1-specific expression of TFL1 rescues the *tfl1* mutant phenotype. The developmentally regulated TFL1 protein trafficking gives rise to the interlayer signaling responsible for the coordinate differentiation of the meristem.

462 T-DNA insertions in sucrose transporters

Jennifer R. Gottwald, Ray F. Evert and Michael R. Sussman

University of Wisconsin, Madison, WI 53706 USA

The movement of carbohydrates from source organs, such as photosynthetic leaves, to sink organs, such as roots and flowers, is one of the major adaptations that allowed plants to evolve organs specialized for different purposes and dependent on each other for resources. A sugar concentration differential along the sieve tubes of the phloem is the driving force for this transport. In species that load phloem apoplastically, sucrose/proton symporters within the sieve element or companion cell plasma membrane are thought to help create this driving force by concentrating sucrose within the loading sieve elements in source organs. In Arabidopsis, these sucrose transporters comprise a family of less than ten members to date. Of these, SUC1 and SUC2 show great homology to each other. Using a reverse genetic screen, we isolated plants with copies of SUC1 or SUC2 containing T-DNA insertions. In *suc1* homozygous plants, no mutant phenotype is evident when plants are grown under normal conditions. *suc2* mutant plants, however, are greatly stunted in growth and slowed in development. They are unable to develop beyond the seedling stage without exogenous sucrose. They rarely produce flowers and never produce viable seed. Their source leaf chloroplasts are packed with starch, suggesting that they are inefficient in exporting sucrose from these leaves. Radiolabelled sucrose applied to these leaves moves through the mutant plants in a different pattern than in the wild type plants. These data suggest that SUC2 is an important part of efficient phloem loading and transport in Arabidopsis.

463 Regulation of Calmodulin Dependent Calcium Pumps

Jeff Harper¹, Amy Curran¹, Ildoo Hwang², Dave Rayle³ and Heven Sze²

¹The Scripps Research Institute, ²University of Maryland, ³San Diego State University
Calcium signals have been implicated in many aspects of plant growth, including the response to stresses such as drought and pathogen attack. The magnitude and duration of a cytosolic Ca signal can be altered by changing the rate of influx or efflux. Ca²⁺-pumps provide an important pathway for Ca efflux. Signals that change their activity can theoretically alter a Ca-signal. A class of plant specific Ca-pumps stimulated by calmodulin have now been identified in the ER, PM and tonoplast of plant cells.

Isoform ACA2, located in the ER membrane of Arabidopsis, can complement a yeast strain (K616) deficient in two Ca-pumps, but only when harboring mutations that disrupt autoinhibition (i.e. make it calmodulin independent). Complementation was observed as the ability to confer growth to K616 on Ca-deficient media. Through mutations we have delineated two regions of the pump that function in autoinhibition. The first (referred to as the autoinhibitor) is a stretch of 32 residues in the N-terminal domain that overlaps with a calmodulin binding site. This region also contains a CDPK phosphorylation site (S45) that blocks calmodulin activation. The second region is in the stalk which connects the ATPase catalytic domain (or head) to the transmembrane domain through which Ca²⁺ ions are translocated. It is not clear if this region of the stalk interacts directly or indirectly with the N-terminal autoinhibitor. The identification of this second region is significant since similarly positioned stalk mutations have been found associated with hyperactivity of proton pumps. A correspondence of regulatory mutations in proton and Ca-pumps suggests that the stalk provides a structural feature important for the regulation of diverse P-type ATPases.

The potential complexity and significance of regulating Ca-pump activity is highlighted by *in vitro* biochemistry showing that while Ca/calmodulin stimulates ACA2 activity, an opposing inhibition results from a second Ca signaling pathway (phosphorylation by CDPKs). *In vivo*, the importance of regulating ACA2 activity is indicated by evidence that the expression of a deregulated Ca-pump in transgenic plants leads to growth defects.

464 Characterization of CAX Genes in Plants

Kendal Hirschi and Toshiro Shigaki

Baylor College of Medicine, Children's Nutrition Research Center, Room 11004, 1100 Bates Street, Houston, Texas 77030

Transporter-mediated Ca²⁺ efflux from the cytoplasm is an important component of plant signal transduction. To elucidate the diversity and role of Ca²⁺/H⁺ in controlling plant cytosolic Ca²⁺ concentrations, homologs of CAX (for CALCIUM EXCHANGER) genes were cloned from corn and Arabidopsis cDNA libraries. The Arabidopsis homolog of CAX (AtHCX1) is 59% identical to CAX1 while the corn homolog of CAX (ZmHCX1) is 56% identical to CAX1 in amino acid sequence. AtHCX1 transcripts appeared to be expressed in all tissues and levels of AtHCX1 RNA increased after Ca²⁺ or Na⁺ treatment. When expressed in yeast mutants defective in vacuolar Ca²⁺ uptake, both AtHCX1 and ZmHCX1 failed to suppress the calcium sensitivity of these strains. We will present data on the structural differences between AtHCX1 and CAX1 which mediate vacuolar Ca²⁺ transport in yeast. Our preliminary results imply that CAX-like genes may have functions in plant ion homeostasis that differ from previously characterized CAX genes.

465 Characterization of *Arabidopsis thaliana* H⁺-ATPase AHA2 in yeast and its implications for a functional role of 14-3-3 proteins in plants

Thomas Jahn and Michael Gjedde Palmgren

The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

The plant plasma membrane H⁺-ATPase generates a proton and electrochemical gradient at the plasma membrane, which is important for nutrient transport across the plant plasma membrane. Activation of proton pumping involves displacement of an autoinhibitory C-terminal domain of the H⁺-ATPase. Alanine-scanning mutagenesis through 87 amino acid residues of *Arabidopsis* H⁺-ATPase AHA2 has revealed the presence of two autoinhibitory regions within the C-terminal domain (Axelsen et al. 1999).

Mutant enzymes that showed improved complementation in yeast showed increased phosphorylation at Thr947, a modification, which resulted in increased binding of yeast endogenous 14-3-3 protein.

Mutation of Thr947 into alanine within the activated mutants resulted in a loss of 14-3-3 binding but did not significantly change the activity compared to the respective single point mutant when measured *in vitro*. However, these double point mutants were no longer able to complement a yeast strain devoid of its endogenous H⁺-ATPase PMA1. Results presented here raise questions about the function of 14-3-3 protein in the regulation of the plasma membrane H⁺-ATPase in plants.

Axelsen et al. (1999) *Biochemistry* 38, 7227-7234.

466 Identification and disruption of plant sulfonylurea receptor-like K⁺ channel regulator involved in K⁺-dependent seedling growth

Ko, Jae Heung, Minjae Kwon, Hochul Yi, and Myeon Haeng Cho

Department of Biology, Yonsei University, Seoul 120-749, Korea

AtSUR, a mammalian sulfonylurea receptor (SUR)-like K⁺ channel regulator identified in *Arabidopsis*, displays the typical structural characteristics of the SUR family, two nucleotide-binding folds including Walker A, B sequences and ABC-signature motif, two membrane spanning hydrophobic domains composed of 15 transmembrane helices and extracellular localized glycosylated N-terminal. AtSUR mRNA is preferentially accumulated in sink tissues such as root, flower, and stem. The knockout mutant disrupted in AtSUR by T-DNA insertion was isolated and shows impaired membrane potential repolarization in response to lowered extracellular K⁺ concentration. When high affinity K⁺ uptake was induced by a low K⁺ treatment, roots of this mutant displayed reduced high affinity potassium (rubidium-86) uptake. Compared with wild type, mutant plants grew poorly on media with a potassium concentration of 100 micromolar or less. In root growth assay, the knockout mutant shows retarded root development and growth in response to low concentrations of K⁺ in growth medium, and also in response to 0.1 M NaCl and 0.2 M Mannitol. AtSUR transcripts were accumulated within 3 hrs in response to hyperosmotic stresses such as 0.3 M NaCl and 0.6 M Mannitol and also by 10 micromolar abscisic acid, a plant hormone involved in water stress response. Also, AtSUR transcripts were accumulated specifically in response to K⁺ treatment among other cations, such as Na⁺, Mg²⁺, Ca²⁺, and Cd²⁺. These results suggested that AtSUR

is involved in K⁺ channel-dependent membrane potential establishment for nutrition uptake and ion homeostasis during osmotic stresses. When K⁺-dependent seedling growth was measured in the absence and presence of light, compared with wild type, mutant seedlings displayed reduced growth such as hypocotyl length and fresh weight in various concentrations of K⁺ in media, and this defects in seedling growth was apparent in etiolated seedlings than in light-grown seedlings. The possible roles of AtSUR in coupling the cellular energy metabolism and plasma membrane potential will be discussed.

This work was supported by grant No. 981-0510-050-2 from the Basic Research Program of the Korea Science and Engineering Foundations.

467 IDENTIFICATION AND FUNCTIONAL ANALYSIS OF ARABIDOPSIS TRANSPORT PROTEINS

Reinhard Kunze², Elisabetta Catoni¹, Beate Saal² Anja Schneider², Karin Schumacher¹, Ulf I. Fluegge² and Wolf B Frommer¹

¹ZMBP, University of Tuebingen, Auf der Morgenstelle 1, 72076 Tuebingen, Germany, and ²Botanical Institute II, University of Cologne, Gyrhofstr. 15, 50931 Koeln, Germany

Solute transport between different plant organs and/or cell compartments is crucial in many biochemical processes. Therefore transport processes are essential for plant development in response to environmental factors and are decisive for the determination of both quality and quantity of crop yield. Analysis of the almost complete Arabidopsis thaliana sequence database reveals that the majority of putative transport proteins with more than four membrane spanning domains are still uncharacterized. The aim of this study is the identification and characterization of Arabidopsis membrane proteins involved in sensing and transport, using the following techniques: 1) Complementation of yeast mutants defective in the uptake of different solutes across the plasma or organellar membranes with an Arabidopsis cDNA library. If not available, the appropriate yeast mutants are constructed by knocking out the respective endogenous yeast transporter genes and the appropriate selection conditions are established. 2) Identification of yet uncharacterized ORFs with more than four membrane spanning domains from the Arabidopsis sequence database. Selected ORFs are expressed in Xenopus oocytes, and the biochemical properties are determined by electrophysiological studies. 3) For functional analysis of the novel membrane proteins in plants, isolation of Arabidopsis knockout mutants as well as studies on antisense RNA expressing lines are carried out. 4) The temporal, spatial and conditional expression patterns of the identified membrane proteins are determined by DNA microarray analysis. cDNA fragments from the genes of interest are amplified by PCR, gridded on glass slides and hybridized with cDNAs from plants grown under various stress conditions.

468 Analysis of an Arabidopsis Ds-tagged albino mutant of which gene encodes a chloroplast TatC homologue: in vivo function of cpTatC in thylakoid protein targeting REIKO MOTOHASHI¹, Takuya ITO¹, Noriko NAGATA², Shigeo YOSHIDA², and Kazuo SHINOZAKI¹

1. Lab: Plant Mol. Biol., Plant Fun. Genome. RIKEN, Tsukuba, Ibaragi 305-0074. 2. Lab: Plant Fun. RIKEN, Wako, Saitama 351-0198

To study functions of nuclear genes involved in chloroplast development in transposon-tagged *Arabidopsis* mutants, we used a two-component transposon system based on the Ac/Ds element of maize as a mutagen. We isolated 2739 Ds-transposed lines and their progenies of F3 plants carrying independent transposition events were screened for mutants with albino or pale green phenotypes.

In this study, we carried out a detailed analysis of four albino mutants 767-1, 2415-1, 1474-1, 1504-1. These mutant phenotypes were caused by Ds insertions based on genetic analyses and southern hybridization. To isolate these mutant genes, we carried out TAIL-PCR to obtain Ds-flanking regions in F2 plants, and determined their nucleotide sequences. Ds inserted in the same *pga2* (for pale green and albino 2) gene that has sequence similarity to *tatC* (for twin-arginine translocation) gene from *E. coli*. We named the *pga2* products as cpTatC according to a chloroplast TatC homologue. The existences of at least three pathways by which nuclear -encoded proteins are targeted to the chloroplast thylakoidal membrane have been suggested. In the Δ pH-dependent (the proton concentration difference) pathway, TatC is a major component of translocator complex. A central role of TatC-type protein is translocation of tightly fold proteins across biological membranes. The *pga2* gene product, cpTatC, is highly hydrophobic and probably contains six transmembrane helices. These four *pga2* alleles contained disrupted cpTatC gene, in which its mRNA was undetectable in mutant leaves. The *pga2* mutants lacked the thylakoid membranes of mature chloroplasts. The *pga2* plastids had similar structures with those of undifferentiated proplastids. We first describe function of plant homologue of the TatC protein in chloroplast development.

469 Assessing ABC Transporter Function by Reverse Genetics

Bosl Noh and Edgar P. Spalding

Dept. of Botany, University of Wisconsin, Madison, WI USA 53706

Proteins in the ABC superfamily of transporters mediate the movement of various substrates across membranes in organisms ranging from prokaryotes to humans. We cloned a new ABC transporter gene from *Arabidopsis* (AtPAC) that clearly falls within the MDR or P-glycoprotein group (36% identical, 57% similar to human MDR1). In mammalian cells, P-glycoprotein is best known for pumping hydrophobic drugs out of resistant tumor cells. The transport activity of AtPAC or any other *Arabidopsis* P-glycoprotein (e.g. *atpgp1*, 51% identical, 69% similar to AtPAC) is not known, but the genetic and physiological studies described here have produced some testable possibilities. Plants were engineered to express promoter:GUS transgenes. The results indicated that AtPAC is expressed in the cotyledons, shoot apex, and root (but not hypocotyl) of light-grown seedlings. The hypocotyl stained very strongly in etiolated seedlings, indicating that light inhibits AtPAC transcription in this organ. To study the function of the AtPAC protein, we isolated a T-DNA insertion mutant by reverse genetics. When homozygous for the insertion, the mutant displayed strongly down-curved cotyledons due in large part to the abnormal extension and angle of the cotyledon petioles, compared with wild-type seedlings. In addition, the first true leaves of the mutant were strongly curled. These morphological features resembled auxin-induced epinasty and could be phenocopied in wild-type seedlings by including IAA or synthetic auxins in the medium. The mutant phenotype was complemented by transformation with the wild-type AtPAC gene. Our working hypothesis is that AtPAC pumps auxin from

cells in tissues that synthesize it. The mutant phenotype may result from the hyperaccumulation of auxin in those cells. In addition to pumping hydrophobic organic molecules, mammalian P-glycoproteins have been suggested to function as regulators of anion channels. The mutant we have obtained will be a useful tool in electrophysiological tests of a channel-regulator function of this P-glycoprotein from Arabidopsis.

470 Polar auxin transport - past, present, future

Klaus Palme

Max-Delbrück-Laboratorium in der Max-Planck-Gesellschaft Carl-von-Linné-Weg 10,
D-50829 Koeln, Germany

Auxins influence a remarkable variety of developmental and physiological processes, but we are still years away to fully understand the molecular basis of auxin action. The application of animal hormone concepts to design experiments to unravel the molecular basis of auxin action has not really advanced our understanding of plant regulation and development. It has been suggested that auxin might be better viewed as a substance which like signals acting in the animal nervous system collates information from various sources and transmits processed information to target tissues. Despite the problems of formulating an entirely convincing conceptual framework for auxin action, the past decade of molecular and genetic research is starting to bear fruits, particularly on the elucidation of the elements of the auxin transport pathway. I will summarize some of the past insights and discuss several genes important for this elusive process.

471 Genetic approaches toward understanding plasmodesmal function

Jennifer Pfluger, Frederick D. Hempel, Insoon Kim, Kyle Sha and Patricia C. Zambryski
University of California Berkeley

Identification of specific structural and/or regulatory components of the plasmodesmal machinery is crucial for understanding the many-faceted role of plasmodesmata throughout plant development. In any given tissue, plasmodesmata have a characteristic size exclusion limit (SEL) for molecular transport between cells. Some mutations in plasmodesmal components would likely result in altered SEL. To identify such mutants, I have initiated a screen for atypical macromolecular movement within the leaf epidermis of Arabidopsis seedlings. Fast neutron and EMS mutagenized lines are being created that express GFP specifically in trichomes via the ATMYB5 promoter, and I will screen for altered patterns of GFP movement from trichome cells.

We are also characterizing two mutants, increased size exclusion limit 1 (ise1) and increased size exclusion limit 2 (ise2), which were identified in an independent screen using 10 kDa F-dextran as probes for increased SEL in embryo defective lines. Both ise1 and ise2 embryos allow cell-cell movement of 10 kDa F-dextran, whereas the SEL in wild type embryos is less than 3 kDa. ise1 and ise2 are embryo defective, with arrest occurring variably from early heart through torpedo stages of embryogenesis. Both mutants can germinate on tissue culture media at low frequency. In these cases ise1 and ise2 make recognizable root- and shoot-like structures but do not produce flowers. Radial tissue organization appears normal although underdeveloped, and overall growth is slower. To facilitate molecular cloning of the affected genes, we have mapped ise1 to the 10 cM region of chromosome 1 on the classical genetic map, and ise2 (allelic to the Meinke lab mutant emb25) to the 100 cM region on chromosome 1.

472 Analysis of sucrose transporter interactions using the split-ubiquitin system
Anke Reinders, Wolf B. Frommer and John M. Ward
Plant Physiology, ZMBP, University of Tübingen, Auf der Morgenstelle 1, D-72076
Tübingen, Germany

The transport of sucrose from source leaves to sinks, such as roots and flowers, is essential for growth and development. Proton-coupled sucrose transporters (SUTs) in the plasma membrane of phloem cells are required for long-distance sucrose transport and phloem function. Three SUT paralogs (SUT1, a high affinity transporter, SUT4, a low affinity transporter, and SUT2, a putative sucrose sensor) have been localized to the sieve element plasma membrane in tomato (Barker et al., 2000; Weise et al., 2000). Physical interactions between SUT proteins and interactions between subdomains of SUT1 were tested using the split-ubiquitin system (Stagljar et al., 1998, PNAS 95: 5187-92). Unlike classical two-hybrid systems, the split-ubiquitin system does not rely on the detection of interactions in the nucleus.

For the lactose permease from *E. coli*, it has been shown that forces between different domains of the protein are able to maintain a functional three-dimensional structure even if the protein is expressed as two separate parts (Bibi & Kaback, 1990, PNAS 87: 4325-29). To test whether similar forces are in effect between domains of the sucrose transporters, SUT1 from potato was expressed in yeast as two separate fragments. These fragments were shown to interact in the split-ubiquitin system. The functionality of the reconstituted transporter was confirmed by yeast complementation. In further experiments, SUT2 was found to interact with both SUT1 and SUT4, supporting a model in which the putative sensor modifies the activity of the other SUT homologs. Experiments to test the functional consequences of these interactions are in progress. It is expected that the split-ubiquitin system will have broad utility for the identification of membrane protein interactions.

Barker, L., Kühn, C., Weise, A., Schulz, A., Gebhardt, C., Hirner, B., Hellmann, H., Schulze, W., Ward, J.M. and Frommer, W.B. (2000) SUT2, a putative sucrose sensor in sieve elements. *Plant Cell*, in press
Weise, A., Barker, L., Kühn, C., Lalonde, S., Buschmann, H., Frommer, W.B. and Ward, J.M. (2000) A new subfamily of sucrose transporters SUT4 with low affinity/highcapacity localized in enucleate sieve elements of plants. *Plant Cell*, submitted

473 Hydrogen peroxide activated calcium channels mediate guard cell ABA signaling, disruption of this response in *gca2* and characterization of a new ABA hypersensitive mutant

Julian Schroeder, Veronique Hugouvieux, Yoshiyuki Murata, Gregor Benning*, Sebastien Thomine, Birgit Klusener, Erwin Grill* and Zhen-Ming Pei
University of California, San Diego, Dept. of Biology, La Jolla, CA 92093-0116,
*Technical University Munich, Lehrstuhl Botanik, Freising-Wethenstephan, Germany
Abscisic acid triggers an increase in guard cell cytosolic calcium. However, direct recordings of ABA-activated CaSUP2+/SUP channel currents have been limited and the upstream activation mechanisms of plasma membrane CaSUP2+/SUP channels in guard cells remain completely unknown. We have now found an unexpected robust

H₂O₂/S₂O₃ activation of Ca²⁺/S⁺ permeable channels in the plasma membrane of *Arabidopsis* guard cells (1). Moreover, H₂O₂/S₂O₃ activation of Ca²⁺/S⁺ channels as well as ABA-induced stomatal closing are impaired in the ABA insensitive recessive mutant, *Igca2*. ABA causes H₂O₂/S₂O₃ production in wild-type and *Igca2* guard cells. Together these data suggest that H₂O₂/S₂O₃ is a second messenger in guard cell ABA signaling, that ABA-activated Ca²⁺/S⁺ channels are important mechanisms for ABA-induced stomatal closing and that the *Igca2* mutant disrupts ABA signaling between H₂O₂/S₂O₃ production and Ca²⁺/S⁺ channels (1). Furthermore, isolation of a new ABA hypersensitive recessive *Arabidopsis* mutant, *Iabh1* will be reported. Isolation of the *IABH1* gene, complementation of the mutant phenotype and functional analyses of *Iabh1* in guard cell signaling point to a new mechanism for global regulation of the sensitivity of ABA signal transduction. Pei, Z.-M., Murata, Y., Benning, G., Thomine, S., Klusener, B., Allen, G., Grill, E. and Schroeder, J.I. (Submitted).

474 Molecular and genetic analysis of the *det3* mutant: A role for the V-ATPase in Ca²⁺-signaling?

Karin Schumacher 1, Gethyn Allen 2, Julian Schroeder 2 and Joanne Chory 3

1 Center for Plant Molecular Biology (ZMBP), University of Tuebingen, FRG; 2

University of California, San Diego; 3 The Salk Institute for Biological Studies, La Jolla

The phenotype of the *det3* mutant was shown to be caused by a reduced activity of the V-ATPase, a highly conserved eukaryotic protein complex whose primary function in endomembrane compartment acidification affects a multitude of cellular processes. In contrast to the detailed structural analysis, knowledge of function and regulation of the V-ATPase is limited. This is, at least partly, due to the fact that in higher eukaryotes, mutants with reduced or abolished V-ATPase function have not been available so far. The *det3* mutant thus offers an excellent opportunity to study V-ATPase function in vivo and to use genetics to approach regulatory mechanisms. One important role for the V-ATPase is in cellular ion homeostasis and we therefore used the CAMEleon reporter construct and a pH-sensitive version of GFP to analyze the effect of reduced V-ATPase activity on pH and Ca²⁺ homeostasis. To gain insight into the regulation of V-ATPase activity, we have used a molecular approach to identify interacting proteins as well as a genetic approach to isolate suppressor-mutants and will present preliminary results.

475 Isolation and molecular characterization of two putative cyclic nucleotide gated ion channels from *Arabidopsis thaliana*

Wolff P, Stracke R, and Palme K

Max-Delbrueck-Laboratorium, Carl-von-Linne-Weg 10, 50829 Koeln, Germany

We have isolated the cDNA of two genes from *Arabidopsis thaliana*, *AtCNBT1* and *AtCNBT2*, coding for members of a new family of plant ion channels. The proteins share a sequence homology of 81 % and are related to the previously described group of putative cyclic nucleotide gated ion channels, *AtCNGC1* to 6, from *A. thaliana* (Koehler et al., 1999). With six transmembrane segments (S1-S6) and a pore forming domain between S5 and S6 their membrane topology resembles those of plant K⁺ inward rectifying, animal K⁺ outward rectifying and cyclic nucleotide gated cation channels. The

pore region is missing the signature sequence typical for K⁺ selective channels. Binding domains for cyclic nucleotides and for calmodulin can be found at the C-terminus. RT-PCR analysis revealed a tissue specific and complementary expression pattern for the two genes. While AtCNBT2 is expressed in guard cells, AtCNBT1 is predominantly active in leaf mesophyll cells in vicinity to the phloem. A C-terminal fusion of AtCNBT1 with GFP was targeted to the plasma membrane in BY-2 cells.

Koehler C, Merkle T, and Neuhaus G 1999. *Plant J.* 18(1), 97-104.

476 Mismatch Repair in Plants: Isolation and Characterization of *Arabidopsis thaliana* MutS Homolog Proteins

Culligan, K.M., and Hays, J.B.

Program in Molecular and Cellular Biology, and Dept. of Environmental and Molecular Toxicology, Oregon State University, Corvallis OR 97331

All eukaryotic organisms examined thus far encode homologs of the eubacterial DNA mismatch repair protein MutS. In comparison to other eukaryotic organisms, little is known about how plants utilize MutS Homolog (MSH) proteins in mismatch-repair systems to combat mutations arising during the process of DNA replication, or to process heterologies formed in DNA following homologous recombination. To address this, we focused on three primary questions: i) Do plants encode homologs of MutS proteins? ii) Do these homologs fall into the same distinct subfamilies seen in other eukaryotes? iii) Do these homologs share conserved biochemical activities with their eukaryotic counterparts? Using sets of degenerate polymerase chain reaction (PCR) primers corresponding to highly conserved MSH protein domains, we amplified and cloned segments of *Arabidopsis* cDNA. Two of these segments encoded conserved portions of *Arabidopsis* MSH genes, and their full-length cDNAs were isolated from *Arabidopsis* cDNA libraries. We further identified two other MSH genes, and their full-length cDNAs were isolated from *Arabidopsis* mRNA using reverse-transcriptase (RT-) PCR. Extensive phylogenetic analyses proved that these four predicted plant-protein sequences clearly fall into the conserved MSH2, MSH3 and MSH6 sub-families of other eukaryotes, and it highlighted a novel feature in plants - the presence of two MSH6-like proteins, designated MSH6 and MSH7. Combinations of *Arabidopsis* AtMSH2, AtMSH3, AtMSH6, and AtMSH7 proteins, products of in vitro transcription and translation, were analyzed for protein-protein interactions. The AtMSH2 protein formed heterodimers with AtMSH6, AtMSH3, or AtMSH7 proteins (designated AtMutS?, AtMutS?, and AtMutS?, respectively) but no other complexes were observed. Furthermore, the abilities of the various heterodimers to bind to mismatched oligoduplexes were measured by electrophoretic mobility-shift assays. Both AtMutS? and AtMutS? share conserved mismatch specificities with their eukaryotic counterparts, but MutS? defines a novel substrate specificity. These data suggest that plants utilize unique mismatch-repair pathways to maintain the integrity of their genomes.

477 Dominant-Negative Mutations in Mismatch Repair Gene AtMSH2 Accelerate Frameshift Mutation in a Microsatellite-Containing Reporter Gene

Jeffrey M. Leonard and John B. Hays

Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR 97331

Evolutionarily conserved DNA mismatch repair (MMR) systems maintain genomic stability by repairing replication errors due to nucleotide misincorporation or polymerase slippage. MMR proteins also antagonize recombination between partially diverged DNA sequences, presumably by recognizing mismatches in recombination intermediates. Although a number of Arabidopsis genes appear to encode orthologs of eukaryotic MMR proteins, little functional evidence has been presented. Since MMR deficiency in other organisms causes microsatellite instability (frequent frameshift mutation at tandem short-nucleotide repeats), we created an in planta functional assay for MMR by inserting an artificial microsatellite (monomeric run of Gs) downstream of the initiating ATG of GUS such that the downstream sequence is out of frame. Subsequent mutations would then revert the gene to functionality. Cells encoding GUS⁺ revertants are scored as blue spots in subsequent whole plant staining. When basal mutation rates were measured by staining plants transformed with the GUS construct, wide line-to-line variation was observed. Nonetheless, plant-to-plant variation within lines was relatively consistent. To perturb the MMR system, we mutated either the conserved ATPase domain or the helix-turn-helix region of AtMSH2 in two separate constructs (mimicking point mutations in yeast MSH2 that conferred dominant negative phenotypes), and transformed them into plants under the control of the CaMV 35S promoter. Plants carrying the mutant AtMSH2 genes were fertile and appeared normal. When crossed into the transgenic lines carrying the GUS microsatellite reporter however, the mutation rate, as measured by the number of blue staining areas, increased 40 to 70 fold above the basal rate measured in the corresponding plants without the dominant negative genes. This large increase in the mutation rate, seen in both leaves and roots, suggests that the overexpressed mutant AtMSH2 protein interfered with the endogenous MMR system, perhaps by blocking access to DNA by functional heterodimers or interfering with multimerization. (Supported by NSF grant MCB 9631048).

478 Genetic control of homologous recombination
Jerzy Paszkowski, Tesfaye Mengiste and Moez Hanin
Friedrich Miescher Institute

Two known pathways are involved in the repair of DNA double-strand breaks (DSBs): non-homologous end joining (NHEJ) and homologous recombination. The proteins involved in the two processes are evolutionarily conserved, but the relative contributions of the two repair processes vary in different biological systems. In higher plants, the prevalent mechanism is NHEJ. In contrast to NHEJ, homologous recombination repairs DNA damage precisely and, thus, the end product of this reaction is predictable. This precision has been exploited for the modification of chromosomal genes (gene targeting) in a number of biological systems. Although gene targeting could be an important technology to study gene function, its effectiveness in higher plants is unsatisfactory due to the low frequency of homologous recombination. We used a genetic approach to identify the molecular components required for intrachromosomal homologous recombination in somatic cells of higher plants. This led to the identification of a protein belonging to the SMC family (structural maintenance of chromosomes). Surprisingly, although loss of SMC function is lethal in other eukaryotes, normal growth of the Arabidopsis mutant in the absence of genotoxic treatments suggests a specialized function of this protein. A further analysis of the DNA repair properties of the mutant

revealed a defect in somatic intrachromosomal homologous recombination. In plants, the low frequency of gene targeting and intrachromosomal recombination is in clear contrast to the efficient extrachromosomal recombination of DNA. Therefore, chromatin accessibility may be a major regulatory step influencing the recombination frequency of chromosomal DNA in vivo. The SMC-like proteins in plants may alleviate hindrance of homologous recombination imposed by suppressive chromatin structure in somatic cells. This hypothesis was supported recently by observed increases in the frequency of homologous recombination due to overexpression of this protein. Thus for the first time it was possible to alter levels of homologous recombination in plants using an endogenous element. This suggests that decoding of further limiting factors will lead to regulation of recombination levels.</p></div>
<div data-bbox="142 298 689 316" data-label="Section-Header"><p>479 Telomerase structure, function, and regulation in Arabidopsis</p></div>
<div data-bbox="142 315 832 351" data-label="Text"><p>Karel Riha¹, Shuxin Ren², Matthew S. Fitzgerald¹, Feng Gao¹, Dorothy E. Shippen ¹, and Thomas D. McKnight²</p></div>
<div data-bbox="142 351 846 387" data-label="Text"><p>¹Department of Biochemistry and Biophysics and ²Department of Biology, Texas A&M University, College Station, TX 77843 USA</p></div>
<div data-bbox="142 386 854 821" data-label="Text"><p>Telomeres, complex nucleoprotein structures at the ends of eukaryotic chromosomes, are required for complete replication of linear chromosomes, to protect chromosomes from exonucleases, and to distinguish natural ends of chromosomes from ends formed by double-stranded breaks. Telomeric DNA is synthesized by telomerase, an unusual reverse transcriptase that carries its own RNA template. We recently characterized a gene encoding the telomerase reverse transcriptase (TERT) from Arabidopsis⁽¹⁾. Plants with a T-DNA insertion in this gene lack telomerase activity, and their telomeres shorten by 500 bp per generation, a rate 10-times slower than seen in telomerase-null mice. The first five generations of telomerase-null plants are phenotypically normal, but sixth and seventh generation plants exhibit distinct defects in vegetative and reproductive development. The long phenotypic lag was expected, given the length of telomeres (2 to 4 kb) and the slow erosion. Defects include "rough" sectors on leaves, altered phyllotaxy in inflorescences, and reduced seed set. Progeny of these plants have more severe phenotypes: leaves are small and asymmetric, there are many small secondary inflorescences, siliques are shorter and often curled, carpels are not completely fused, and seed set and germination efficiency are reduced. The severity of the defects correlates with the extent of telomere depletion, suggesting that loss of telomeres results in cell cycle arrest and exhaustion of proliferation potential. We are also interested in the regulation of telomerase, particularly during the transition from the vegetative phase to the reproductive phase. Telomerase is not expressed in leaves, but it is abundant in flowers. We are screening activation-tagged lines to identify mutants that ectopically express telomerase in their leaves. Such mutants may result from activation of a gene that controls telomerase expression during development. We have found two mutants with abundant telomerase activity in their leaves. Neither mutation is near the TERT gene, suggesting that novel genes have been activated in these two lines.</p></div>
<div data-bbox="142 837 547 855" data-label="Footnote"><p>⁽¹⁾ Fitzgerald et al., 1999. PNAS 96,14813-14818.</p></div>
<div data-bbox="142 872 836 891" data-label="Section-Header"><p>480 B and C floral organ identity functions require SEPALLATA MADS-box genes</p></div>

Soraya Pelaz*, Gary S. Ditta*, Elvira Baumann+, Ellen Wisman+, and Martin F. Yanofsky*

*Department of Biology, University of California at San Diego, La Jolla California 92093-0116, USA,

+ Max-Planck-Institut für Züchtungsforschung, Carl-von-Linnè-Weg 10, 50829 Köln, Germany

The differentiation of four distinct organ types of the flower (sepals, petals, stamens and carpels), each of which has been proposed to represent a 'modified leaf', is the result of the coordinate action of the floral organ identity genes. The landmark ABC model of flower organ identity was proposed in order to understand how these genes act during flower development. This simple model provides a conceptual framework for explaining how the individual and combined activities of the ABC genes produce the four organ types of the typical eudicot flower. We show that the activities of the B and C organ identity genes require the activities of three closely related and functionally redundant MADS-box genes, SEPALLATA1-3 (SEP1-3). We used PCR-based screens to identify loss-of-function alleles of SEP1, SEP2 and SEP3. Although single mutants produce only subtle phenotypes, triple mutant Arabidopsis plants lacking the activities of all three SEP genes produce flowers in which all organs develop as sepals. Thus SEP1-3 define a class of organ identity genes that is required for development of petals, stamens and carpels. In addition, the sep1-3 triple mutant flowers are indeterminate, suggesting that besides their organ identity roles, the SEP genes are required to prevent the indeterminate growth of the floral meristem. A model for the interactions between the SEPALLATA proteins and the previously described ABC proteins will be presented.

481 Regulation of the L1-layer-specific gene expression in Arabidopsis thaliana Mitsutomo Abe, Taku Takahashi, Yoshiyumi Komeda

Division of Biological Sciences, Graduate school of Science, Hokkaido University
The shoot apical meristem (SAM) of Arabidopsis thaliana constitutes the tunica of L1 and L2 and the corpus represented by L3 cells. Although recent genetic and morphological studies have identified several key genes involved in the regulation of the SAM maintenance, little is known about the molecular mechanisms for layer-specific gene expression at the SAM. The Arabidopsis thaliana PDF1 (PROTODERMAL FACTOR1) gene is specifically expressed in the L1 layer and the protoderm of organ primordia. Previously we have shown that the 1.5 kb region immediately upstream of the PDF1 coding region contains essential cis-regulatory elements for the L1-layer-specific expression. In order to further define the cis-regulatory sequences, various PDF1 promoter fragments were fused to the β -glucuronidase (GUS) gene and assayed for the resulting patterns of expression in transgenic plants. Our analysis revealed that the 149-bp sequence in the PDF1 promoter between -260 and -111 is required for spatially restricted expression. Electrophoretic mobility shift assays demonstrated the presence of protein factors in whole cell extracts from flower bud clusters that bind to this region. We identified a likely cis-acting sequence in this region, which is also present in the upstream region of other L1-layer-specific genes such as ATML1, FDH, and LTP1. These results suggest that trans-acting factors responsible for the L1 specific gene expression are shared by these genes.

482 The HASTY gene encodes a protein with similarity to the importin/exportin family of nucleocytoplasmic transport receptors

Milo J. Aukerman, Krista M. Bollman, Abby Telfer and R. Scott Poethig
University of Pennsylvania

Loss-of-function mutations in the Arabidopsis HASTY (HST) gene cause a variety of different phenotypes, including smaller leaves that curl upward at the margins, accelerated expression of adult traits, and smaller, less developed roots. HST may also affect abaxial/adaxial organ polarity, as we observe occasional crabs claw-like defects in *hst* mutant flowers, including partially unfused carpels and ectopic ovules. To further assess the potential role HST may have in the abaxial/adaxial polarity pathway, we are currently analyzing double mutant combinations between *hst* and *crabs claw*, *filamentous flower* and *kanadi*. In order to understand the molecular mechanisms underlying the pleiotropic *hst* mutant phenotype, we have cloned the HST gene using a map-based approach. The HST gene product shows amino acid sequence similarity to the importin/exportin family of nucleocytoplasmic transport receptors. To assess the subcellular localization of the HST protein, we have created a translational fusion between the HST gene and the *E. coli* β -glucuronidase (GUS) reporter gene, and produced transgenic plants expressing this HST/GUS fusion protein. Staining of these plants for GUS activity indicates that the HST/GUS fusion protein accumulates within cells in a ring-like pattern coincident with the nuclear membrane. This result is consistent with a role for HST in nucleocytoplasmic transport, either as an exportin, an importin, or both. We are currently analyzing proteins which interact with HST in a two-hybrid assay in an attempt to identify potential HST "cargo" - i.e., proteins which are transported into or out of the nucleus by the HST protein.

483 sidekick of *clavata* is an Enhancer of *clv1* Defects in Floral and Shoot Meristems
Catherine C. Baker and Elliot M. Meyerowitz

California Institute of Technology, 1201 E. California Blvd, Pasadena, CA 91125

The shoot apical meristem (SAM) consists of a dome of cells which gives rise to the entire above-ground structure of the plant. The size and shape of the meristem are genetically regulated, presumably via control of cell proliferation and cell differentiation (into stem, leaves, flowers, etc.). The CLAVATA signaling pathway has been shown to restrict meristem size; loss-of-function mutations in either CLV1 or CLV3 result in enlarged shoot and floral meristems. We have searched for additional elements of this pathway or parallel pathways by using an activation-tagging screen in an intermediate *clv* mutant background (*clv1-1*). Among the mutants which have been identified in this screen is a strong enhancer of *clv1-1*, which we have named sidekick of *clavata* (*soc*). *soc*; *clv1-1* plants have SAMs about 1 mm in diameter, extra carpels in the flowers, and abundant fifth-whorl tissue. *soc* shows incomplete dominance in a *clv1-1* background.

484 The Genetics of Nectary Development in Arabidopsis

Stuart F. Baum, Yuval Eshed and John L. Bowman

Section of Plant Biology, U.C. Davis, Davis, CA 95616

Nectaries secrete nectar which is used as a food source for animal pollinators. In Arabidopsis flowers, the nectary is composed of a ring of tissue in the third whorl with glands subtending the abaxial side of stamen filaments. Nectary glands are composed of

densely cytoplasmic cells containing starch surrounded by an epidermis possessing a reticulate patterned cuticle. Nectar is secreted through modified stomata located at the apex of the gland. CRABS CLAW (CRC) is required but is not sufficient for nectary development and all other known nectary gene expression is CRC dependent. In wild-type plants, nectaries are associated with stamens but they can develop independently of stamens. In pi-1, ap3-3, ag-1, ap2-2 ag-1 and ap2-2 pi-1 ag-1 mutant plants, organs other than stamens occupy the third whorl and yet nectary glands are observed, albeit the size and morphology are altered. Over-expressing the B class genes in a wild type or ap2-2 mutant background creates flowers with multiple whorls of stamens but nectary glands are still only observed in the third whorl. Since nectaries are not dependent upon the ABC genes, they are free to move about the flower over evolutionary time. In sup-1 in which there are multiple third whorls, nectary glands are present in all third whorls. Ectopically expressing UFO causes a reiteration of the third whorl and not merely an activation of B class genes in the fourth whorl as evidenced by the presence of nectary glands. In addition, ectopically expressing B class genes in a lfy-6 ufo-2 double mutant restores petals and stamens but not nectaries. Nectary development is dependent on intrinsic factors associated with the third whorl.

485 Epidermal Patterning in Arabidopsis Cotyledons

Gregory J. Bean and Judith L. Croxdale

University of Wisconsin, Madison WI USA 53706

Two types of specialized cells normally occur on the epidermis of *Arabidopsis thaliana*, trichomes and stomatal guard cells. We examined stomatal distribution in selected trichome mutants to determine whether the mutations altered two-dimensional epidermal patterning on the cotyledons. Cotyledons provide an excellent model for study because they lack trichomes, permitting unobstructed views of the surface. We imaged entire abaxial surfaces of mature cotyledons by scanning electron microscopy for statistical analysis of stomatal pattern. Lines used included a wild type (Nossen), which we compared to mutants for trichome absence (gl 1-1), trichome clustering (try 240), and stomatal formation (cyd and f-100). We found that the 2-dimensional pattern of stomata differed between Nossen and gl 1-1, and that frequency of stomata (number per unit area) was greater in Nossen than it was in gl 1-1. We conclude that mutations in the trichome pathway affect stomatal patterning in cotyledons, even though they have no trichomes. While these findings may seem counterintuitive, they may indicate that epidermal patterning in all leaves is affected by mutations in specialized epidermal cells.

486 A subtilisin-like serine protease involved in the regulation of stomatal density and distribution

D. Berger, U. von Bieberstein, and T. Altmann

Max-Planck-Institut fuer molekulare Pflanzenphysiologie, Am Muehlenberg 1, 14476 Golm, Germany

Stomata are specialized cellular structures in the epidermises of aerial plant organs that control gas exchange (H₂O release and CO₂ uptake) between leaves and the atmosphere by modulating the aperture of a pore flanked by two guard cells. Stomata are non-randomly distributed, and their density is controlled by endogenous and environmental factors. To gain insight into the molecular mechanisms regulating stomatal distribution,

Arabidopsis thaliana mutants with altered stomatal characteristics were isolated and examined. The *sdd1-1* mutant exhibits a two- to fourfold increase of stomatal density and the formation of clustered stomata (i.e., stomata that are not separated by intervening pavement cells), whereas the internal leaf architecture is not altered. Analysis of developing leaf epidermis revealed a role of the SDD1 gene in the regulation of (1) the fraction of protodermal cells forming stomatal initials, (2) the degree of higher order stomatal complex formation, and (3) in the control of cell division orientation in higher order meristemoids. The SDD1 gene was identified by map based cloning (Berger and Altmann, 2000). It encodes a subtilisin-like serine protease related to prokaryotic and eukaryotic proteins. Expression analysis through mRNA in situ hybridization and promoter-GUS fusions revealed activity of the SDD1 gene in guard cell mother cells and young, developing guard cells. In analogy to the function of other eukaryotic subtilases, SDD1 is proposed to act as a processing protease involved in the mediation of a signal emanating from guard cell mother cells and young, developing guard cells that controls the development of cell lineages forming stomatal complexes.

Berger, D. and Altmann, T. (2000) A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in *Arabidopsis thaliana*. *Genes Dev.*, in press.

487 Tortifolias lead the way to straight growth

Buschmann, H., Fabri, C.O., Hauptmann, M., Schaeffner, A.R.

GSF Research Center for Environment and Health, Institute of Biochemical Plant Pathology, D-85764 Neuherberg, Germany

Tortifolia (*tor*) mutants have been identified by their striking twisted growth of petioles during cell expansion phase. The recessive *tor1* and *tor2* mutants consistently develop a right-handed torsion, whereas the semi-dominant *tor3* develops a left-handed twist. Additive and synergistic genetic interactions among these mutations suggest that primarily independent pathways are involved. In addition, *tor* mutants exhibit different orientations of root slanting. Therefore, they may be classified as circumnutation mutants. Based on these phenotypes we hypothesize that circumnutation or similar endogenous movements may be essential in order to establish straight growth that is achieved by a continuous control of deviations from a straight axis in a dynamic process. In *tortifolia* mutations different components of this system may be damaged or missing. The TOR loci were mapped to chromosome 4 (TOR1), 1 (TOR2), and 5 (TOR3) using ARMS markers. Positional cloning of TOR1 revealed a gene expressed in leaves, roots, stems, and flowers. Its amino acid sequence is not homologous to other known plant proteins. However, TOR1 constitutes a small gene family in *Arabidopsis*.

488 Asymmetric leaves1 mediates axis specification and stem cell fate in *Arabidopsis*
Mary Byrne¹, Mark Curtis¹, Ross Barley², Juana-Marie Arroyo¹, Maitreya Dunham¹,
Andrew Hudson², and Rob Martienssen¹

¹ Cold Spring Harbor Laboratory, 1 Bungtown Rd, Cold Spring Harbor, New York 11724, USA. ² Institute of Cell and Molecular Biology, University of Edinburgh, United Kingdom

The shoot apical meristem (SAM) comprises undifferentiated stem cells at the tip which divide slowly to give rise to peripheral cells from which leaf founder cells are recruited. Founder cells divide much more rapidly, initiating outgrowth of organ primordia and simultaneously establishing polarity along the proximodistal, dorsoventral and mediolateral axes. We are interested in the mechanism by which stem cells and founder cells interact in the meristem, and the contribution of these interactions to leaf determination and patterning. SAMs are characterized by expression of knox (Knotted-like) homeobox genes, and down regulation of these genes in founder cells on the flank of the SAM is an early event in leaf initiation. Down regulation of knox genes in leaf primordia is maintained by the myb domain transcription factor PHANTASTICA in *Antirrhinum* and its ortholog ROUGH SHEATH2 in maize (Waites et al. 1998. *Cell*: 93, 779-789; Timmermans et al. 1999. *Science*: 284, 151-153; Tsiantis et al. 1999. *Science*: 284, 154-156). The *Arabidopsis* mutant asymmetric leaves1 (*as1*) is disrupted in specification of the leaf proximodistal axis resulting in lobed and dissected leaves with a characteristic asymmetry. We have found that *AS1* encodes the *Arabidopsis* ortholog of *phan* and *rs2*. We also show, by molecular and genetic interactions, that *AS1* negatively regulates the knox genes *KNAT1* and *KNAT2* and is, in turn, negatively regulated by the knox gene *SHOOT MERISTEMLESS (STM)*. This genetic pathway defines a mechanism for differentiating between stem cells and founder cells within the shoot apical meristem.

489 The ARGONAUTE gene is needed for adventitious rooting and secondary growth in the hypocotyl of *A.thaliana*

I. Camus¹, H. McKhann¹, C. Bréart¹, I. Barlier¹, C. Garcion¹, C. Benning² and C.Bellini¹

¹ Laboratoire de Biologie cellulaire, INRA, Versailles, France. ² Dept. of Biochemistry, Michigan State University, USA

The *AGO1* of *A. thaliana* gene encodes a putative 115 KDa protein (*EMBO J.* 1998,17, 170-180) belonging to the zwiille eIF2C piwi stingRDE1 family conserved from plants to vertebrates (*EMBO J.* 1998, 17, 1799-1809; *Gene* 1998, 211, 187-194; *Genes Dev.*, 1998, 12, 3715-3727; *Genetics*, 1999, 151, 749-760; *Cell* 1999, 99, 123-132). The rabbit protein eIF2C has been implicated in translation initiation. RDE1 has been shown to be involved in gene silencing and RNAi in the nematode *C. elegans*. These results suggest that this gene family may have a role in the translational or post translational gene expression. In northern-blot experiments the *AGO1* mRNA was constitutively expressed in all organs and during seedling development from the cotyledon stage until the 6/10 leaves stage. Similarly, western blot analysis showed that the *AGO1* protein is also present in the different organs of the plants and at different stages of development. Mutation in the *AGO1* gene dramatically affects plant architecture and leads to seedlings with unexpanded cotyledons, narrow leaves and a unique stem with abnormal inflorescence bearing sterile flowers. More precise characterization of the phenotype showed that *ago1* mutants are not able to develop adventitious roots from the hypocotyl or other aerial organs in presence of exogenous auxin whereas roots respond normally. Double mutants between *ago1* and *superroot 1*, a natural auxin overproducer (*Plant Cell*, 1995, 7, 1405-1419) showed that *ago1* was partially epistatic on *sur1*. The double mutants developed more lateral roots than *ago1*, like *sur1*, but they were unable

to develop adventitious roots either from the hypocotyl or from leaf and cotyledon explants unlike *sur1*. Further characterization also showed that *ago1* mutants did not show secondary growth either in the hypocotyl or in the stem. Because adventitious rooting and secondary growth are both controlled by auxin, these results suggest that among the genes which expression may be controlled by AGO1 some of them could be involved in auxin dependant developmental processes.

490 *eli1*: a link between cell expansion and secondary cell wall formation in *Arabidopsis*

Ana Cano-Delgado and Mike Bevan

John Innes Centre

Plant cell walls play a central role in cell growth and morphogenesis. The formation of a secondary wall is restricted to particular cell types, such as the xylem cells, highly lignified cells that provide mechanical and transport functions to the plant. To identify genes involved in controlling secondary cell wall formation we screened for mutants with altered patterns of lignification and altered xylem development in the primary root of *Arabidopsis thaliana*. Three different complementation groups have been established. One of these mutants, *eli1* exhibited ectopic lignification of cells throughout the plant that never normally lignify. *eli1* has been characterized in detail. Xylem cells in *eli1* were misshapen and failed to differentiate into continuous strands, causing a disorganized xylem. *eli1* exhibited abnormal cell expansion resulting in a stunted phenotype.

Ultrastructural analysis of *eli1* cell walls using an anti-lignin antibody showed that ectopic lignin deposition occurs with an altered secondary wall formation. We observed that related ectopic lignification phenotypes appear in other mutants with altered cell expansion, such as the *lit*, *rsw1* (at conditional temperature) and the *det3*. These results together with the double mutant analysis of *eli1* with other cell expansion mutants suggested a mechanism that senses cell size and controls secondary wall deposition. Using SSLPs the *eli1* mutation was found to map in chromosome 5. The identification of the gene responsible for the *eli1* mutation will provide an important insight into the molecular control of the switch between cell expansion and secondary cell wall formation during plant morphogenesis.

491 Genes identified from mutational and QTL analysis affecting organogenesis in *Arabidopsis* tissue culture

Andrew Cary¹, Shyam J. Uttamchandani¹, Todd Vision², Owen A. Hoekenga¹, Rafael Smets³, H. A. Van Onckelen³ and Stephen H. Howell¹

¹Boyce Thompson Institute and ²USDA-ARS Center for Bioinformatics and Comparative Genomics, Cornell University, Ithaca, New York 14853 USA and ³University of Antwerp, Antwerpen, Belgium

Pioneering studies by Skoog and Miller in the 1950s demonstrated that one can direct the developmental fate of tissues regenerating in culture by the combined action of plant hormones. We have set up a system based on regeneration procedures developed by Valvekens to study shoot and root formation in root explants of *Arabidopsis*. In this system, root explants are preincubated on callus induction medium (CIM) during which they acquire the competency to produce shoots when shifted to a cytokinin-rich shoot induction medium (SIM) or roots on an auxin-rich root induction medium (RIM). We

have searched for factors that affect the shoot and root formation using classical genetics and quantitative trait analysis in recombinant inbred (RI) lines. We identified four non-complementing mutants, called increased organ regeneration (ire) mutants, that form green callus or shoots at sub-optimal concentrations of cytokinin on SIM. One of these is allelic to a previously identified pom1/erh2 mutant. Although the mutants were selected for more robust regeneration on sub-optimal concentrations of cytokinin, they are neither hypersensitive to cytokinin nor overproducers of biologically active forms of the hormone. Instead, the mutants predispose root explants to respond more robustly to organ regeneration stimuli conferred by either cytokinin or auxin. Recombinant inbred (RI) lines derived from crosses of Col and Ler ecotypes show considerable variation in their capacity to form organs, and we have used that variation to identify major quantitative trait loci (QTLs) influencing organ formation. We found that some QTLs affect either shoot, green callus, or root formation while others affect more than one process. New assortments of characters in the RI lines have given rise to lines that are more robust than either parent in the formation of shoots or roots. These lines are being used to study the changing patterns of gene expression during shoot and root development.

492 TCP1, the orthologue of the CYCLOIDEA gene, reveals a new domain of positional identity in Arabidopsis

Cubas, P. and Martínez-Zapater, J. M.
INIA/CNB

The CYCLOIDEA (CYC) gene plays a key role in generating the floral asymmetry of *Antirrhinum majus*. This gene is expressed in the dorsal (adaxial) part of floral meristems from very early stages of development and affects cell division in this region.

CYCLOIDEA is a putative transcription factor containing a TCP domain, sequence that may play a role in nuclear targeting, DNA binding and dimerization. We are characterising the function of TCP1, the Arabidopsis orthologue of CYC. TCP1 is expressed in the adaxial region of axillary meristems. This suggests that the positional cues controlling TCP1 transcription are equivalent in all axillary meristems regardless the structure they will form -either flower or shoot- and supports the classical view of flowers as modified shoots. It also reveals the underlying asymmetry of axillary meristems and provides a scenario of how floral asymmetry may have evolved. To further analyse TCP1 function we have characterised Arabidopsis lines containing insertions in TCP1 and transgenic plants in which TCP1 ORF is overexpressed in sense or antisense orientation. Phenotype of plants from these lines will be presented and discussed.

493 Characterization of Two Receptors Functionally Redundant With CLV1

Brody J. DeYoung¹, Amy E. Trotochaud¹, Katherine J. Schrage¹, Kanu Patel², Jonathan D. Jones², Paul Muskett², Caroline Dean², and Steven E. Clark¹

¹University of Michigan, Department of Biology, Ann Arbor, MI, USA 48109-1048;

²John Innes Centre, Norwich, UK NR4 7UH

The proper regulation of the meristem is crucial for normal plant development. Our goal is to understand the molecular mechanism of meristem regulation. Arabidopsis plants with mutations at the CLAVATA loci (CLV1, CLV2, and CLV3) accumulate undifferentiated cells in the shoot and floral meristems. CLV1 codes for a receptor protein kinase, while CLV2 codes for a receptor protein lacking a kinase domain. The

current hypothesis is that CLV1 and CLV2 are receptors that are present in the plasma membrane of cells in the meristem and act to perceive an extracellular signal and transmit it into the cell, eventually altering the transcription of genes in the nucleus. CLV1 has been shown to exist in two protein complexes in wild type Arabidopsis plants. The larger (450 kD), active complex requires CLV1, CLV2, and CLV3 for formation. The 450 kD complex also contains a protein phosphatase that regulates the level of phosphorylation of CLV1 and thus its signaling capacity, and a Rho-like GTPase. The smaller (185 kD), inactive complex is thought to contain only CLV1 and CLV2.

Two proteins, CLV1-like 14 (CLL14) and CLL15, have been identified that show a significant sequence identity to CLV1. Because of inconsistencies between *clv1* lesions and phenotypic severity, we have hypothesized that these receptors are functionally redundant with CLV1. Here, CLL14 and CLL15 are shown to be associated with large molecular weight complexes similar to the CLV1 complexes. In addition, a reduction in expression of CLL14 and CLL15 in transgenic plants causes a *clavata*-like phenotype. Taken together these preliminary results support the hypothesis that CLL14 and CLL15 function in a manner similar to CLV1. Insertional mutants for CLL14 and CLL15 have been generated to further test this hypothesis. The mutation in CLL14 consists of a T-DNA insertion in the region of the gene that codes for the kinase domain. The mutation in CLL15 consists of a transposon insertion 5' of the putative transmembrane domain encoding sequence.

494 Cloning and characterization of REVOLUTA and related genes

John F. Emery, Yuval Eshed, and John Bowman

Section of Plant Biology, UC Davis, One Shields Avenue, Davis, California

We have cloned the REVOLUTA gene from a T-DNA enhancer trap line. It encodes a homeodomain-leucine zipper putative transcription factor and is a member of the Arabidopsis thaliana class III homeodomain family1. It is expressed in both vasculature and adaxial regions of developing lateral organs. Its two closest relatives, AtHB9 and AtHB14 have similar expression patterns. These patterns are complementary to those of three members of the Yabby gene family, FILAMENTOUS FLOWER (FIL), YABBY2, and YABBY3 which direct the development of abaxial regions in lateral organs2. Since *revoluta* mutants strongly interact with *fil* mutants, we believe these genes are involved in the development of adaxial characters of lateral organs.

1. Sessa, G. et al., Plant Molecular Biology 38: 609-622 (1998).
2. Siegfried, K. et al., Development 126: 4117-4128 (1999).

495 PASTICCINO1, a large FKBP involved in the control of cell proliferation and differentiation

JD. Faure, R. Cowling, T. Masson, Y. Harrar and C. Bellini

Laboratoire Biologie Cellulaire, INRA 78026 Versailles Cedex, France

The PAS1 gene from Arabidopsis encodes for a 70kDa protein with similarity to the FKBP52 family. PAS1 is characterized by 3 repeats of a FK506-binding domain and three tetratricopeptide repeats (TPR) (Vittorioso et al. 1998 Mol. Cell. Biol. 18: 3034-3043). PAS1 shows rotamase (PPIase) activity which is inhibited by FK506. PAS1 is expressed in the highly dividing tissues corresponding to the plant "stem cells"

(meristem). We have isolated 2 mutants in PAS1 gene which represent the first inactivation of a large FKBP gene in higher eukaryotes. Disruption of PAS1 gene leads to a severe developmental phenotype with altered embryo, leaf and root development. These developmental defects are correlated with uncoordinated cell divisions and cell dedifferentiation (Faure et al. 1998 Dev. 125: 909-918). Cyclin dependent kinase (cdc2) and mitotic cyclin (cyclin B1) are ectopically expressed in proliferative area suggesting that the cell cycle machinery is up-regulated in pas1 mutant. Application of cytokinins, a class of plant growth factors involved in promoting cell divisions leads to cell proliferation and loss of cell adhesion in pas1 tissues which is reminiscent of the structure of animal teratomas. The expression of the PAS1 gene is up-regulated by cytokinins in wild type tissues suggesting that PAS1 would normally either inhibit cell division or induce cell differentiation like anti-oncogenes in animals. Several PAS1 interacting proteins, were isolated through 2-hybrid screening and classified according the PAS1 domains they interact with. Their putative function in the pasticcino phenotype will be discussed.

496 Rescue of the stm mutant phenotype by expression of STM mRNA in the peripheral zone: implications for nonautonomous STM action

Anita G. Fernandez, Jeff Long, Rebecca E. Joy, and M. Kathryn Barton

Laboratory of Genetics, University of Wisconsin-Madison, 445 Henry Mall, Madison, WI 53706

The SHOOTMERISTEMLESS (STM) gene product is required for development of the Shoot Apical Meristem (SAM) and all of its derived products including leaves, stems, flowers, and seeds. The stm- phenotype is dramatic: plants germinate with root, hypocotyl, and cotyledons but no SAM. STM mRNA and protein are expressed throughout the developing SAM from early embryogenesis until senescence. This expression pattern is consistent with STM's role of establishing and maintaining the SAM.

The 3.5 KB genomic region upstream of the STM translation start codon drives expression of the GUS reporter in only the peripheral zone (PZ) although STM mRNA and protein are normally found in both the peripheral zone and the central zone (CZ). Little is known about the relative roles of STM in the PZ versus the CZ. In order to understand this better, the STM cDNA was expressed from this "partial" (PZ only) promoter in stm-11/stm-11 plants. Surprisingly, these transformed stm-11/stm-11 individuals, which normally make just two cotyledons, were indistinguishable from wild type. They flowered and set seed normally. Thus expression of STM in a subset of its normal domain yields complete rescue of our strongest stm hypomorph.

In order to understand how this could happen, expression of STM mRNA and protein was analyzed in rescued homozygous stm-11 embryos. The STM protein was found at wild-type levels in both the CZ and the PZ. In contrast, the STM mRNA was significantly reduced in the CZ. This result is consistent with the STM protein, expressed in the PZ, moving into the CZ in rescued embryos. We are pursuing further experiments to investigate this possibility, as well as the role of such movement in the normal development of the SAM.

497 The Genetics of Root Hair Morphogenesis in Arabidopsis thaliana

J. L. Foreman, P. Mylona, P. Linstead & L. Dolan
John Innes Centre, Norwich, Norfolk, UK

Model organisms are used to study complex developmental processes because they allow the use of a number of experimental approaches to elucidate their molecular basis. Root hair development in *Arabidopsis thaliana* is a useful model system for studying general growth and development of plant cells. During differentiation of the root, specialized cells in the epidermis (trichoblasts) form root hairs, tubular shaped outgrowths. After elongation in the axis of the root has ceased, a bulge forms at the apical end of trichoblasts, from which a tip-growing hair emerges. Hairs can attain lengths of 1mm and growth arrest is accompanied by vacuolation of the hair tip. Mutants with defects in root hair morphogenesis have been identified. A class of these mutants cease growth soon after initiation. In these "bleb" mutants initiation of a hair begins, but growth prematurely aborts before tip growth occurs. These mutants help elucidate the genetic and molecular basis of root hair morphogenesis. Complementation tests have shown that our screens have identified five genes involved in the process. Phenotypic characterization and double mutant analysis has begun to help identify the roles of these genes in growth. Two of these five genes involved in root hair morphogenesis have been cloned. Recent data will be presented. Molecular characterization will help to determine their exact roles in root hair morphogenesis. Analysis of these new "bleb" mutant lines will help elucidate the molecular understanding of growth, differentiation and development in plants.

498 Characterization of *corona* (*cor*), an enhancer of *clv*

Kirsten A. Green, Rebecca B. Katzman, Lita Po Yu, and Steven E. Clark
University of Michigan, Ann Arbor, MI

The shoot meristem maintains a pool of undifferentiated cells which gives rise to all of the above-ground organs. *SHOOTMERISTEMLESS* and *CLAVATA* loci function in tight balance controlling the transition of undifferentiated cells toward organ formation. *stm* mutants are unable to maintain undifferentiated cells resulting in early cessation of organ development. In contrast, *clv* mutants are characterised by an accumulation of undifferentiated cells resulting in enlarged floral meristems and club-shaped gynoecia. To identify other genes involved in the CLV pathway, *clv1-1* seeds were EMS mutagenized, and M2 progeny from individual M1 plants were screened for enhanced and suppressed phenotypes of the *clv1-1* phenotype. *cor clv* double mutants have massively enlarged shoot meristems and terminate organ formation shortly after flowering. This cessation of organ formation is not due to inhibited growth since the meristem continues to enlarge, but is caused by the inability to form organ primordia. Thus, the *cor* mutation, enhances the organ formation defects of *clv* mutants. Later in development, a breakdown in the meristem of *clv cor* doubles occurs such that the formation of carpeloid-like organs occurs across the entire meristem. *cor* single mutants have only very slightly larger than wildtype shoot meristems, and occasionally exhibit altered phyllotaxy. They are difficult to distinguish from wildtype plants. We propose that *COR* promotes organ formation in a separate pathway from that of the CLV loci. In-situ analysis suggest that the expression pattern of *STM* is altered as well as moderately upregulated in *cor clv3-2* double mutants as compared to *clv3-2*, and thus *COR* may be acting in the *STM* pathway. Preliminary evidence also suggests that the expression of *LEAFY* (*LFY*), a meristem identity gene involved in both floral and shoot

meristem development, is downregulated in young *clv3-2 cor* doubles. However, later in development, there are strong pockets of LFY expression prevalent throughout the carpeloid-like organs. This, together with evidence that STM limits expression of LFY, suggests that COR may have a role upstream of STM and LFY and lends support to the idea that STM is an intermediary, linking LFY to the meristem regulatory pathway.

499 Characterization of the FRINGE gene family of putative glycosyl transferases in *Arabidopsis*

Nathaniel P. Hawker and John L. Bowman

University of California Davis, Davis, CA 95616

We have begun the task of characterizing the members of a large gene family in *Arabidopsis*. This family of putative glycosyl transferases has some homology to the FRINGE gene of *Drosophila*, hence our name for the family. In animals, FRINGE genes are involved in modulating cell to cell signalling. I will present expression data for several members as well as phylogenetic analysis of the FRINGE family. The precise expression patterns of the members analyzed thus far, suggest that these genes may have specific roles within plant development.

500 In vivo analysis of development in the shoot apex with OCM

Hettinger, J.1, M.de la Pena Mattozzi², W.Myers², M.Williams², A.Reeves¹,

R.Parsons¹, R.Haskell², D.Petersen², R.Wang² and J.Medford¹

Colorado State Univ¹, Fort Collins CO 80523, Harvey Mudd College², Claremont CA 91711

We have developed and used a new technology, optical coherence microscopy (OCM), which allows in vivo visualization of underlying tissues and cells in plants. OCM allows rapid, repetitive and non-destructive collection of data. The current instrument allows us to image up to 1 mm deep in living tissues, resolving structures less than 5 μm in size, with a typical collection time of 5-6 minutes. The basis of OCM is the inherent light-scattering properties of biological tissues and cells. Photons back-scattered from small volume elements (voxels) are collected, measured, assembled and the voxels quantitatively false-colored to form a three-dimensional image. Analysis uses custom-designed visualization software that allows the images to be cropped or sliced and/or the opacity of the images decreased so that underlying tissues can be seen. OCM images are false colored with a spectrum where high light-scattering regions typically appear red whereas regions of low light-scattering typically appear blue. Theoretical studies suggest that cells with high light-scattering will have large, active nuclei and prominent organelles. These studies agree well with our experimental work that shows that in large cells, high light-scattering is produced from nuclei, intermediate light-scattering is produced from cytoplasm, and little light-scattering originates from the vacuole and cell wall. In planta, all analyses to date indicates that high light-scattering regions in *Arabidopsis* correlate with regions undergoing developmental processes. OCM images are highly predictable and hence provide a type of endogenous developmental marker. For example, developing leaf primordia typically have high light-scattering in regions corresponding to the incipient blade. In regions such as the shoot meristem, the pattern of light-scattering is dynamic, changing as often as 1-2 hours. Pattern formation in the shoot

apex of known Arabidopsis mutants (e.g., *clavata1*, primordia timing) is aberrant and the OCM images of these mutants show distinct light-scattering patterns.

501 A critical role of sterols in embryonic patterning and meristem programming revealed by the *fackel* mutants of *Arabidopsis thaliana*

Jyan-Chyun Jang,¹ Shozo Fujioka,² Masao Tasaka,³

¹Department of Horticulture and Crop Science, The Ohio State University, Columbus, Ohio 43210 ²RIKEN, Wako-shi, Saitama 351-0198, Japan ³Graduate School of Bioscience, NAIST, Ikoma Nara 630-0101, Japan

We report here a novel Arabidopsis dwarf mutant, *fackel-J79*, whose adult morphology resembles that of brassinosteroid-deficient mutants but also displays distorted embryos, supernumerary cotyledons, multiple shoot meristems, and stunted roots. We cloned the *FACKEL* gene and found that it encodes a protein with sequence similarity to both the human sterol reductase family and yeast C-14 sterol reductase and is preferentially expressed in actively growing cells. Biochemical analysis indicates that the *fackel-J79* mutation results in deficient C-14 sterol reductase activity, abnormal sterol composition, and reduction of brassinosteroids (BRs). Unlike other BR-deficient mutants, the defect of hypocotyl elongation in *fackel-J79* can not be corrected by exogenous BRs. The unique phenotypes and sterol composition in *fackel-J79* indicate crucial roles of sterol regulation and signaling in cell division and cell expansion in embryonic and post-embryonic development in plants.

502 Genetic and biochemical characterization of *CLV2*

Sangho Jeong and Steven E. Clark

University of Michigan, Department of Biology, Ann Arbor, MI 48109-1048

Arabidopsis plants homozygous for recessive mutations at the *CLAVATA* loci (*CLV1*,² and *3*) accumulate undifferentiated cells in shoot and flower meristems resulting in enlarged meristems with abnormal organogenesis. *CLV1* encodes a receptor-like kinase (RLK) with extracellular leucine-rich-repeat (LRR) domain and *CLV2* encodes a receptor-like protein (RLP) which has an extracellular LRR domain and transmembrane domain but no appreciable cytoplasmic domain. *CLV1* exists as a disulfide-linked 185-kD multimer *in vivo*, which forms a 450-kD active signaling complex requiring *CLV3*. Lacking a cytoplasmic signaling domain, *CLV2* has to interact with other proteins to signal across the plasma membrane. The *CLV1* RLK is a good candidate because both *CLV1* and *CLV2* genes function in the same pathway. *CLV1* protein does not accumulate in *clv2* mutants although mRNA level is normal, supporting a model that *CLV1* and *CLV2* form a disulfide-linked heterodimer. To have direct evidence for the heterodimer, we introduced c-terminal myc-tagged version of *CLV2* into *clv2* mutants. Because the fusion construct complemented *clv2* mutation, now we can test physical association of *CLV2* and *CLV1* by co-immunoprecipitation. One unique feature of *clv2* mutants is that the flower phenotype is suppressed under short day photoperiod condition. Because *clv2* null mutants are suppressed, it is the underlying biological mechanism that is sensitive to photoperiod. Using various flowering time mutants for double mutants analysis, we tested whether specific genes are involved in the suppression. Interestingly, the photoperiod-dependent flowering pathway was found to be critical. This pathway may function as one of the 'input' pathways for *CLV* signaling pathway after detecting

photoperiod changes. While CLV1, CLV2 and CLV3 are in the same signaling pathway for flower meristem development, only *clv2* is suppressed, indicating that the suppression occurs at the same level as CLV2. The simplest hypothesis would be that there is an RLP that can substitute for CLV2 function under short day condition. We have identified possible mutants in genes required for the suppression under short days. To test whether CLV1/CLV3 are involved in the suppression, we are measuring CLV1 and CLV3 accumulation in *clv2* mutants under long day and short day conditions.

503 A Screen for Embryo Patterning Mutants Using SHOOTMERISTEMLESS as a Molecular Marker for the Developing Shoot Apical Meristem

Rebecca E. Joy, Thaddeus R. Hoening, and M. Kathryn Barton

Laboratory of Genetics, University of Wisconsin, Madison

Mutants defective in establishing fundamental patterns of gene expression during Arabidopsis embryogenesis have proven difficult to find. This may be because such mutants arrest during embryogenesis. To test this idea, we have screened among embryo arrest mutants for those that either ectopically express or fail to express a SHOOTMERISTEMLESS (STM) reporter gene. The STM reporter gene consists of 5' sequences flanking the STM coding sequence fused to the GUS gene. Beginning at the late globular stage of embryogenesis, this reporter marks a small group of cells that will become the shoot apical meristem; most pattern defects are expected to alter the placement or development of this domain. The STM reporter line was mutagenized and 1695 independently isolated embryo defective mutants were identified and scored for their pattern of STM reporter expression. 182 of these mutants fail to express the STM reporter - these are currently being tested for their ability to express a root-specific marker. 69 mutants express the STM reporter in ectopic locations. Five of these (blueberry1, 2, 3 and button mushroom 1 and 2) arrest at the globular stage and express the STM reporter throughout the entire globular embryo but not in the suspensor. These mutants define five different complementation groups. Finally, one unique mutant, bobber, expresses the STM reporter in the entire upper hemisphere of the globular stage embryo including the presumptive cotyledon primordia. bobber mutants arrest at the globular stage. In situ hybridization analysis of the UFO and ANT transcripts in bobber mutants indicates that the BOBBER gene is not required for general gene repression. Positional cloning of this gene is in progress.

504 FASCIATA complex has replication-dependent chromatin assembly activity and is involved in regulation of gene expression in apical meristems

Hidetaka Kaya, Kei-ichi Shibahara¹, Yasushi Kobayashi, Masaki Iwabuchi, Bruce Stillman¹, and Takashi Araki¹

Department of Botany, Graduate School of Science, Kyoto University, Kyoto, JAPAN,
¹Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA

FASCIATA (FAS) genes are involved in proper organization of shoot and root apical meristems. FAS1 and FAS2 are homologs of genes for p150 and p60 subunit of Chromatin Assembly Factor-1 (CAF-1), respectively. FAS1, FAS2, and a third component, AtMSI1, form a complex, and the FAS complex has replication-dependent chromatin assembly activity in vitro. Yeast CAF-1 has shown to be involved in inheritance of epigenetically determined chromosomal states that maintain gene

expression/repression states. This implies that the FAS complex has a similar role in Arabidopsis. We examined the expression of two representative marker genes in apical meristems.

WUSCHEL (WUS) gene is expressed in a small group of cells in the center of the shoot apical meristem, and regulates stem cell fate of overlying cells. In *fas* mutants, WUS expression domain was laterally and apically expanded. Extent of expansion differed from meristem to meristem, and in a given meristem, was not uniform. Ectopic expression of WUS may be responsible for the fasciation phenotype.

SCARECROW (SCR) is expressed in quiescent center (QC), cortex/endodermis initial and daughter cells, and endodermis. In *fas1* mutant, SCR::GFP expression was missing in a single or a group of cells, or ectopically detected in a small group of cells adjacent to the authentic cells of expression, such as cortex or columella initials. The pattern was variable among mutant roots, and older roots tended to deviate more from the wild-type pattern. These were consistent with the pattern abnormality observed in *fas* root meristems.

FAS1 gene is expressed in patches of cells in embryo and shoot apical meristem. FAS1 promoter is able to confer G1/S phase-preferential expression on GUS in cell cycle-synchronized BY-2 cells. These suggest that at least some aspects of FAS1 function are associated with replication.

These results together are consistent with the view that FAS complex may regulate gene expression via chromosomal states in meristem cells.

505 The KANADI gene promotes abaxial fate in Arabidopsis leaves
Randall Kerstetter, Krista Bollman, Alex Taylor, and Scott Poethig
University of Pennsylvania

The leaves of most plants exhibit striking differences between adaxial (upper) and abaxial (lower) sides. Some of these differences are apparent from the time the leaf primordium first arises on the flanks of the shoot apical meristem whereas others appear later in development. The distribution of trichomes is a useful feature that distinguishes ad- and abaxial leaf surfaces in Arabidopsis. Juvenile leaves produce only adaxial trichomes, adult leaves produce both ad- and abaxial trichomes, and bracts progressively lose adaxial trichomes. Loss-of-function mutations in KANADI (KAN) result in the ectopic appearance of trichomes on the abaxial surface of juvenile leaves. Misexpression of KAN leads to a variety of polarity defects including the formation of ectopic abaxial tissue, production of radialized leaves, and the failure of the shoot apical meristem to initiate lateral organs. Expression analysis indicates KAN localizes to the abaxial side of developing leaf primordia. A functional KAN gene is required for the proper expression of a KAN promoter:GUS fusion. These observations indicate a role for KAN in promoting abaxial leaf identity. Here, we report the molecular characterization of the KAN locus and show that it is a member of a family of plant genes related to "myb-related" transcription factors. Genetic interactions between KAN and the YABBY gene FILAMENTOUS FLOWER will also be described.

506 BELI : Ovule Morphogenesis and beyond?
Kumuda Kushalappa, Dietmute Godt, Mark Pidkowich and George Haugh
Department of Botany, University of British Columbia, Vancouver, BC V6T1Z4

Arabidopsis BEL1 encodes a homeodomain transcription factor that is a key component of the regulatory cascade directing ovule morphogenesis. Although loss-of-function alleles of BEL1 only affect ovule development, our recent work suggests BEL1 may control other aspects of plant development. By yeast two hybrid analyses we have shown that BEL1 protein interacts with specific members of Arabidopsis Knotted like sub family of homeobox genes (KNAT), at least some of which have key roles in meristem function. Consistent with this BEL1 gene is transcribed in additional tissues outside of ovule where it may function in combination with these interacting proteins. Interestingly, BEL1 null mutations do not result in any obvious phenotypic effects in these tissues. Further expression analyses of some of BEL Like Homeobox genes (BLH) demonstrate a substantial overlap with that of BEL1 in plant organs outside of ovule. It is thus possible, BEL1 and BLH genes encode redundant functions, a hypothesis that could explain why BEL1 null mutation has no effect in other tissues out side where it is expressed.

507 WEREWOLF, a MYB-Related Protein in Arabidopsis Is a Position-Dependent Regulator of Epidermal Cell Patterning

Myeong Min Lee and John Schiefelbein

Department of Biology, University of Michigan

The formation of the root epidermis of Arabidopsis provides a simple and elegant model for the analysis of cell patterning. A novel gene, WEREWOLF(WER), is described here that is required for position-dependent patterning of the epidermal cell types. The WER gene encodes a MYB-type protein and is preferentially expressed within cells destined to adopt the non-hair fate. Furthermore, WER is shown to regulate the position-dependent expression of the GLABRA2 homeobox gene, to interact with a bHLH protein, and to act in opposition to the CAPRICE MYB. These results suggest a simple model to explain the specification of the two root epidermal cell types, and they provide insight into the molecular mechanism used to control cell patterning.

508 The Establishment and Regulation of GLABRA2 Gene during Embryogenesis of Arabidopsis thaliana

Yan Lin, John Schiefelbein

University of Michigan

A position-dependent pattern of epidermal cell types is produced during root development in Arabidopsis thaliana . This pattern is reflected in the expression pattern of GLABRA2 (GL2) , a homeobox gene that regulates cell type differentiation in the root epidermis. In gl2 mutants, an excessive number of hair cells develop, indicating that GL2 is required for non-hair cell differentiation. GL2 promoter::GFP fusions, GL2 promoter::GUS fusion, and in situ hybridization were used to explore the expression pattern of GL2 in both embryos and seedlings of Arabidopsis thaliana . It was found that Arabidopsis root hair cells, which are shorter and located at the clefts between two cortical cell walls, do not exhibit GL2 gene expression. Similarly, cells located outside anticlinal cortical cell walls in the hypocotyl, exhibit reduced cell length, form stomata, and lack GL2 expression. GL2 gene expression is identified at the heart stage of Arabidopsis embryogenesis, and a similar position-dependent pattern of GL2 -promoter activity was discovered in both hypocotyls and roots in embryos and young seedlings. The role of the TRANSPARENT TESTA GLABRA (TTG) gene, the WEREWOLF

(WER) gene and the CAPRICE (CPC) gene was also explored. These results suggest that a specific expression pattern of GL2 gene is established and regulated during Arabidopsis embryogenesis.

509 The MONOPOLE gene encodes a GATA-factor involved in the regulation of cell fates at the basal pole of the early embryo

Wolfgang Lukowitz & Chris Somerville

Carnegie Institution, Department of Plant Biology, 260 Panama Street, Stanford, CA 94305, USA

In Arabidopsis, formation of the root meristem is initiated at the basal pole of the 16-cell embryo. The embryonic root is formed through an almost invariant sequence of cell divisions involving the uppermost suspensor cell as well as the adjacent cells of the embryo proper. From a large-scale mutant screen for embryos with altered morphology we have recovered four recessive allelic mutations, designated MONOPOLE, which interfere with this process. The basal cells of MONOPOLE mutant embryos divide aberrantly producing fewer and irregularly arranged daughter cells. As a consequence, no root primordium is recognizable by anatomical criteria. However, MONOPOLE mutants are capable of forming a functional root later in embryogenesis. A molecular marker for the quiescent center of the root is expressed in the central cells of the embryo suggesting that the embryonic root is initiated ectopically. We have cloned the MONOPOLE gene in a map-based approach and found that it encodes a protein with high similarities to transcriptional regulators of the GATA family. We are presently determining the expression pattern of MONOPOLE RNA in the early embryo.

510 Exploring the role of PINHEAD and PINHEAD-LIKE genes in Arabidopsis shoot development

Karyn Lynn and M. Kathryn Barton

Program in Cellular and Molecular Biology, University of Wisconsin-Madison, 445 Henry Mall, Madison, Wisconsin 53706

Flowering plants develop nearly all shoot organs postembryonically from the shoot apical meristem (SAM). The SAM maintains a reservoir of slowly dividing, pluripotent cells. While some cells differentiate into new organ primordia, others remain in the SAM to replenish those cells lost through organ initiation. The Arabidopsis PINHEAD (PNH) gene (also called ZWILLE) has a role in maintaining the reservoir of cells in the SAM. In pinhead mutants the primary SAM, which is formed during embryogenesis, often terminates concurrently with the development of a single leaf-like organ at the shoot apex. Furthermore, whereas wild-type plants form additional SAMs postembryonically in their leaf axils, pinhead mutant axils often lack SAMs. Thus PNH is also important for postembryonic SAM initiation. PNH encodes a novel protein of unknown biochemical function. It is a member of an Arabidopsis gene family that includes ARGONAUTE1, which is partially redundant to PNH based on genetic analysis, and several uncharacterized loci. PNH-LIKE gene families are found in other eukaryotic organisms as well. The few reported mutations in these genes lead to a variety of defects including failure to carry out self-renewing germ-line stem cell divisions in *Drosophila piwi* mutants, dorsoventral and posterior patterning defects in *Drosophila aubergine* mutants, and suppression of post-transcriptional gene silencing in the *C. elegans rde-1* mutant.

Most recently, Qde-2, a *Neurospora crassa* gene required for post-transcriptional gene silencing was identified as a PNH-LIKE gene. In *Arabidopsis*, PNH mRNA is expressed in developing vascular tissue (although pinhead mutants lack overt vascular defects). It is also expressed continuously in the SAM and on the adaxial, but not abaxial, sides of lateral organ primordia. This expression pattern reveals a new region of positional identity in the shoot apex, unifying the SAM and the adaxial sides of organs surrounding it with respect to expression of PNH mRNA. Data will be presented from recent experiments exploring the role of PNH in meristem formation and the function of some other members of the PNH gene family in *Arabidopsis*.

511 WOODEN LEG regulates vascular patterning during root development in

Arabidopsis

Ari Pekka Mähönen (1), Martin Bonke (1), Leila Kauppinen (1), Marjukka Riikonen (1), Philip N. Benfey (2), Ykä Helariutta (1)

(1) Institute of Biotechnology, POB 56, FIN-00014, University of Helsinki, Finland; (2) Department of Biology, New York University 1009 Main, 100 Washington Sq E, New York, NY, 10003

The conductive system of the vascular plants consists of xylem, phloem and (pro)cambium. We are studying the genetic control of vascular patterning during root development in *Arabidopsis*. We have determined the cell lineage relationships of the vascular tissue in the root and we are analyzing mutations with altered vascular pattern in the root. A recessive mutation in the WOODEN LEG (WOL; Scheres et al. 1995: Development 121, 53-62) gene results in a loss of a set of asymmetric cell divisions and an enlarged domain of xylem differentiation, indicating that WOL plays a central role in the vascular patterning of the root. Further characterization of the mutant with developmental markers as well as the current status of the positional cloning effort will be presented.

512 PRS determines the fate of cells at the lateral ends of floral meristem and floral organs

Noritaka Matsumoto, Kiyotaka Okada

Department of Botany, Graduate School of Science, Kyoto University

The lateral organs of plant have two axes, the abaxial-adaxial axis and lateral axis. Position of floral organ in floral meristem could be determined in relation to the two axes. Cell differentiation, followed to the floral organ development, is also affected by the conceptual abaxial-adaxial axis and lateral axis in the organ primordia. Four sepals are developed in the abaxial, adaxial and lateral sides of a floral primordia of wild type. In the pressed flower (prs) mutant, however, flower had defects in two sepals at the lateral position, which were transformed to small sepals, to filamentous organs or sometimes lacked. The adaxial and abaxial sepals and the other floral organs develop to as same size as in wild type. In addition, the specifically ordered cells at the margin (at the lateral end) of sepals disturbed at the margin of the abaxial and adaxial sepals of the mutant. We have cloned the PRS gene by the positional cloning method. PRS gene was in the middle of the chromosome 2, and encodes a protein containing 244 a.a., with a WUS-like homeodomain at the N terminus. It is suggested that the PRS gene functions as a transcription factor. The experiments of in situ hybridization with the PRS gene probe

showed unique expression patterns. The PRSgene was expressed in cells at the lateral ends of the floral primordia, where the primordium of the lateral sepals are formed. The PRSgene is also expressed in several cells at the margins of the young sepals, petals and stamens. These expression pattern of the PRSgene is mostly consistent with the phenotype of the mutant, except the expression in petals and stamens. Phenotype of the prsmutant and the expression pattern of the PRSgene suggest us that fate of young cells in floral primordia and in organ primordia is specified depending on the abaxial-adaxial axis and the lateral axis.

513 Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis

Yukiko Mizukami and Robert L. Fischer

Dept. Plant & Microbial Biol., Univ. California Berkeley

The control of cell proliferation during organogenesis plays an important role in initiation, growth, and acquisition of the intrinsic size of organs in higher plants. To understand the developmental mechanism that controls intrinsic organ size by regulating the number and extent of cell division during organogenesis, we examined the function of the Arabidopsis regulatory gene AINTEGUMENTA (ANT). Previous observations revealed that ANT regulates cell division in integuments during ovule development and is necessary for floral organ growth. Here we show that ANT controls plant organ cell number and organ size throughout shoot development. Loss of ANT function reduces the size of all lateral shoot organs by decreasing cell number. Conversely, gain of ANT function, via ectopic expression of a 35S::ANT transgene, enlarges embryonic and all shoot organs without altering superficial morphology by increasing cell number in both Arabidopsis and tobacco plants. This hyperplasia results from an extended period of cell proliferation and organ growth. Furthermore, cells ectopically expressing ANT in fully differentiated organs exhibit neoplastic activity by producing calli and adventitious roots and shoots. Based on these results, we propose that ANT regulates cell proliferation and organ growth by maintaining the meristematic competence of cells during organogenesis.

514 Analysis of Cell Identity in Guard Cell Mutant Shabondama

Tsuyoshi Nakagawa (1), Jeanette A. Nadeau (2), Fred D. Sack (2)

(1) Research Institute of Molecular Genetics, Shimane University, Matsue 690, Japan; (2) Department of Plant Biology, Ohio State University, 1735 Neil Avenue, Columbus OH 43210

Stomata are composed of a pair of guard cells and control gas exchange in plants. Because the guard cells have highly specialized metabolism and morphology, it is attractive to study the developmental program of the guard cells. The shabondama1 mutant make round shaped cells instead of kidney shaped guard cells, suggest that division of guard mother cells are prevented in this mutant. To analyze the identity of these cells, we introduced the KAT1 promoter-GUS reporter constructs into the shabondama1. The KAT1 is a gene for potassium channel and known to be expressed in mature guard cells. We found GUS staining in round shaped cells of shabondama1. These results indicate that round shaped cells have character of guard cells in some part.

515 Functional analysis of SCARECROW and SHORT-ROOT genes in root radial pattern formation

Keiji Nakajima and Philip Benfey

Department of Biology, New York University, 1009 Main Bldg., 100 Washington Square East, New York, NY 10003

The Arabidopsis root has a simple and highly consistent radial pattern of cell files, which makes it a tractable system for studying pattern formation in plant development. Mutation in either the SCARECROW (SCR) or SHORT-ROOT (SHR) gene results in a single cell layer between stele and epidermis, in place of the normal two cell layers, endodermis and cortex. The SCR and SHR genes are expressed in both embryo and mature roots. Since root radial pattern is first established in the embryo and then perpetuated in mature roots by well organized cell divisions, the persistent expression of SCR and SHR made it difficult to interpret their roles in radial pattern formation. SCR and SHR encode proteins belonging to the GRAS family of putative transcription factors. Expression of a GFP::SCR fusion protein under the native promoter resulted in a complete rescue of the mutant phenotype, and the GFP fluorescence was found in the nucleus of the cell layers where the SCR gene is transcribed, indicating that it functions in the nucleus. To analyze their roles in the embryo and mature roots separately, we applied the glucocorticoid receptor fusion system, which allowed us to induce SCR and SHR gene functions through nuclear translocation. Upon treatment with a steroid hormone, these transgenic plants partly rescued the mutant phenotypes, apparently depending on the timing of the treatment. Detailed analysis using these inducible genes will be presented.

516 Reverse genetical approaches for studying the function of GLABRA2 (ATHB-10)
Yohei Ohashi¹, Atsuhiko Oka¹, Ida Ruberti², Giorgio Morelli³, and Takashi Aoyama¹
¹Kyoto University, Japan. ²Centro di studio per gli Acidi Nucleici, Italy. ³Istituto Nazionale della Nutrizione, Italy

GLABRA2 encodes a homeodomain-leucine zipper transcription factor (ATHB-10) which regulates the trichome development and the root hair formation. Genetical studies have revealed that ATHB-10 functions during the morphogenesis of trichomes. It is, however, still unclear what kind of cell biological process the gene is concerned with. To address the question, we attempted to analyze the gene function with transgenic Arabidopsis plants expressing ATHB-10 in different manners. As a first step, we examined a 35S promoter-driven ATHB-10 gene. The transgene, however, couldn't complement the defect of the gl2 mutation. Moreover, it conferred a gl2-like phenotype on some transgenic lines of the wild-type background, suggesting that the construct caused co-suppression of the ATHB-10 endogene. Since most of the lines didn't express the transgene at detectable levels in the Northern analysis, we concluded that the 35S promoter is not appropriate for expressing the ATHB-10 function. We tried other promoters including the authentic one and analyzed effects of the transgenes on the trichome development. The results will be shown and discussed.

517 A homeobox gene MOB interacts with the CURLY LEAF gene in patterning homeotic expression in floral development

Carolyn K. Ohno and Elliot M. Meyerowitz

Division of Biology, 156-29, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125

Position-effect variegation and transcriptional gene silencing are examples of chromatin modulated transcription that are observed in many organisms including plants. The recessive mutant modifier of B function (*mob*) was identified as an enhancer of a weak curly leaf (*clf*) mutant allele in a genetic screen for chromatin modifiers of floral homeotic transcription in *Arabidopsis*. The *mob clf* double mutant plants display a mosaic partial homeotic transformation in the flowers that suggests ectopic expression of the B function gene, *PISTILLATA* (*PI*), which is required to specify petal and stamen organs. Indeed, *MOB* is necessary for the precise tissue specific initiation and maintenance of expression of the B function genes, as well as for proper transcription of a reporter gene directed by *PI* regulatory sequences. The sequence of the *MOB* locus has been determined by a map-based positional cloning approach. The *MOB* gene encodes a protein with a homeodomain similar in structure to *Drosophila* Zeste protein. In a screen for phenotypic enhancers of the *mob* mutant an additional gene that also shows homology to regulators of chromatin structure has been identified.

518 Mutations in the *YODA* gene interfere with elongation of the zygote and development of its basal daughter cell into a suspensor

Parmenter, D.L.1, Lukowitz, W.1, Roeder, A.2, Somerville, C.R.1

1 Dept. of Plant Biology, Carnegie Institute of Washington, Stanford, CA 94305, USA 2

Dept. of Biology University of California at San Diego, La Jolla, CA 92093 USA

After fertilization, the wild-type *Arabidopsis* zygote elongates about three-fold and divides asymmetrically, resulting in a small apical and a large basal cell. While the apical cell gives rise to the embryo proper, the basal cell forms a file of cells known as the suspensor. The uppermost suspensor cell contributes to the root meristem. All other suspensor cells are extra-embryonic and eventually degenerate. The examination of EMS-induced mutants with abnormal suspensor development led to the identification of fifteen mutant alleles of a single complementation group named *YODA*. Mutations in *YODA* severely interfere with elongation of the zygote. The basal cell of *YODA* embryos is significantly smaller than in wild type and does not appear to develop into a suspensor. Instead, *YODA* embryos develop as cone-shaped structures lacking any morphologically recognizable distinction between embryonic and extra-embryonic tissue. Most *YODA* embryos do not germinate. Occasionally, however, mutant seedlings are obtained which develop into severely dwarfed and sterile adults. We have taken a map-based approach towards cloning *YODA* and have identified a physical interval of about 100 kb containing the gene. We are presently analyzing candidate transcription units in this area.

519 Regulation of Shoot Development by the *SERRATE* and *ENSALADA* Genes

Michael J. Prigge and D. Ry Wagner

Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

Genes that regulate organogenesis can be identified through the characterization of mutations that either impede organ formation or alter organ morphology. Mutations in the *SERRATE* (*SE*) and *ENSALADA* (*ENS*) genes result in complex phenotypes affecting the initiation and elaboration of lateral organs. Interestingly, the acquisition of embryo

bilateral symmetry is also perturbed when the maternal parent is homozygous for either mutation. Consistent with a role in transcriptional regulation, the SE locus was found to encode a zinc-finger protein whose mRNA accumulates in the meristem and in newly initiated organ primordia. Based on double mutant analysis results, the SE and ENS genes function in a common pathway that is distinct from those of CLAVATA1, TOUSLED, LUENIG, and FASCIATA1. Furthermore, the synergistic *se fas1* and *ens fas1* double mutant phenotypes indicate that the SE/ENS and FAS1 pathways perform redundant functions during organogenesis. A model consistent with our data proposes that the SE and ENS gene products coordinate changes in gene expression in primordia emerging from the meristem.

520 CHIMERIC FLORAL ORGANS (CFO) is a new mutation affecting floral organ development

Marcela Rojas-Pierce, Cristina Reinaga-Peña and Patricia S. Springer

Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124

We have identified an Arabidopsis mutant, chimeric floral organs (*cfo*), that shows defects in floral organ development. This semi-dominant mutation arose in a gene trap transposon tagging population, but is not linked to a transposable element. Plants homozygous for the mutation produce chimeric floral organs, with sepals, petals and stamens being most affected. The *cfo* mutation also affects floral organ number, and filamentous structures are often produced in mutant flowers. The *cfo* phenotype resembles that of the unusual floral organs (*ufo*) mutation. In order to examine genetic interactions between CFO and previously characterized genes, we have combined the *cfo* mutation with mutations in UFO, FUSED FLORAL ORGANS2 (FFO2) and FILAMENTOUS FLOWER (FIL). In each case, the phenotype of the double mutant is more severe than either single mutant phenotype. The CFO gene maps very close to FIL in chromosome II. The phenotypic characterizations and the mapping of this gene will be presented.

521 Characterization of a Novel Gene Encoding a Putative Single Zinc-finger Protein, ZIM, Expressed during the Reproductive Phase in Arabidopsis thaliana

Masahito SHIKATA, Akiko NISHII, Miho TAKEMURA, and Takayuki KOHCHI

Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-0101, Japan

By differential screening of an arrayed normalized cDNA library from the inflorescence apex in Arabidopsis, a cDNA clone having a deduced amino acid sequence with a motif for a zinc finger was isolated as one of the genes expressed specifically in the reproductive phase. The deduced protein has a modular structure with a putative single C2-C2 zinc-finger motif distantly related to a GATA-1-type finger, a basic region with a sequence resembling a nuclear localization signal, and an acidic region. The gene seemed to have been formed by the exon-shuffling during its molecular evolution, since individual domains are encoded by discrete exons. RNA gel blot analysis showed its expression in shoot apex and flowers in the reproductive phase. The gene was named ZIM for Zinc-finger protein expressed in Inflorescence Meristem. The nuclear localization of ZIM was detected using GFP as a reporter. The transcription activation by

ZIM was analyzed in vivo by using ZIM proteins fused to the DNA binding domain of GAL4 as effectors, and the luciferase gene under control of promoter with GAL4 UAS as reporters. These results suggest that ZIM is a putative transcription factor involved in inflorescence and flower development. Functional analyses of ZIM with knockout lines and modified transgenic lines are in progress.

522 ET22, A Member of a New Plant Specific Gene Family, Functions in Organ Separation

Bin Shuai, Cristina Reynaga-Peña, Patricia Springer

Dept. of Botany and Plant Sciences, University of California, Riverside

By using an enhancer/gene trap transposon tagging system, we have isolated a gene, ET22 that appears to play a role in defining organ boundaries. The expression pattern of ET22 has been characterized based on GUS activity in the DsE-tagged line. During early embryo development, GUS activity is localized throughout the embryo, and becoming localized to the root tip and shoot apex as the embryo matures. In seedlings and mature plants, GUS activity is primarily limited to organ boundaries: the basal boundaries of leaf primordia; the base of each coflorescence, mature leaf, and floral organ; and the junctions between lateral and primary roots. Transgenic plants with overexpression or antisense suppression of ET22 show morphological defects that demonstrate its importance in organ separation and meristem function. The predicted amino acid sequence of ET22 does not show similarity to previously characterized proteins. Its amino terminus, however, shares sequence similarity with many hypothetical genes from Arabidopsis, and expressed sequence tag (EST) sequences from other plant species, indicating the importance of this conserved domain in plant kingdom.

523 grv2: A viable embryonic mutant characterized by the presence of enlarged and highly vacuolated cells at the apex

Rebecca A. Silady^{1,2}, Wolfgang Lukowitz¹, Patrick Sieber³, Chris Somerville¹

¹ Carnegie Institute of Washington, Department of Plant Biology, ² Department of Biological Sciences, Stanford University, ³ University of Zurich

The grv2 mutant was identified in a screen in Arabidopsis for mutants with altered morphology at the globular stage of embryogenesis. The grv2 mutant is characterized by enlarged cells in the apex of the embryo, first observed at the two cell stage in cleared whole mount specimens. The enlarged cells appear to have a single nucleus with an abnormally large vacuole. The embryo recovers by the late heart stage at which time the enlarged cells are no longer observed. Despite this mutant phenotype, the grv2 mutant develops into a relatively normal adult plant except that it is apparently agravitropic. This research project focuses on characterizing and mapping the grv2 mutant in order to gain insight into embryonic development.

524 Molecular genetic analysis of corymbosa2, an Arabidopsis mutant with corymb-like inflorescence

Mitsuhiro Suzuki, Taku Takahashi, Yoshibumi Komeda

Division of Biological Sciences, Graduate School of Science, Hokkaido University, N10,W8,Sapporo 060-0810, Japan

Arabidopsis thaliana is a typical rosette plant with distinct vegetative and reproductive growth phases. The reproductive growth is characterized by the elongation of stem internodes in parallel with the production of flower buds. To understand a molecular basis underlying the inflorescence shoot architecture, we have identified *corymbosa2*, a mutant with corymb-like inflorescences similar to *erecta*. Our anatomical study revealed that the corymb-like phenotype of the tip of the inflorescence in *crm2* mutants is mainly caused by the increase in the number of flower buds at the tip. On the other hand, the time to flower initiation appeared to be normal in *crm2*. Our data suggested that the delay in the beginning of stem elongation and in the development of flowers lead to the increase in the number of young flower buds at the tip. In addition, *crm2* plants also showed a reduced fertility. Morphological analyses of double and triple mutants with *er*, *tfl1*, and *clv1*, suggested that CRM2 is a new class of gene that acts independently of other genes related to the inflorescence development. Genetic analysis indicated that the *crm2* locus is mapped between the CAPS markers, *g13838* and *GA5*, on chromosome 4. Further, fine mapping experiments delimited the locus to a 80kbp region which was covered with 2 BAC clones. Isolation of the gene is currently in progress and will be presented.

525 An enhancer trap line associated with a D class cyclin gene in *Arabidopsis*
Kankshita Swaminathan, Yingzhen Yang, Natasha Grotz and Thomas Jack
Department of Biological Sciences, Dartmouth College, Hanover, NH 03755 USA
In yeast and animals cyclins have been demonstrated to be important regulators of cell cycle progression. One class of cyclins, the D class cyclins, is important for progression through G1 phase of the cell cycle. In *Arabidopsis*, four D class cyclins have been isolated and characterized (*CYCD1;1*, *CYCD2;1*, *CYCD3;1*, *CYCD4;1*). Here we describe the characterization of a fifth D class cyclin gene, *CYCD3;2*, from *Arabidopsis*. This cyclin gene is adjacent to the insertion site in an enhancer trap line (line 5580) that exhibits expression in young vegetative and floral primordia. In line 5580, T-DNA is inserted in the first exon of the *CYCD3;2* gene; in homozygous 5580 plants *CYCD3;2* RNA is not detectable. Even though *CYCD3;2* gene function is eliminated, homozygous 5580 plants do not exhibit an obvious growth or developmental phenotype suggesting functional redundancy. To ensure that *CYCD3;2* encodes a functional cyclin we expressed it in a yeast strain deficient in G1 cyclin activity and found that *CYCD3;2* was able to rescue the yeast mutant. Based on Northern and in situ hybridization, *CYCD3;2* RNA is expressed in the shoot apical meristem and developing vegetative and floral primordia.

526 rabbit ears (*rbe*) mutant reveals that petal development in the abaxial and adaxial sides is differently regulated
Seiji Takeda, Noritaka Matsumoto, Kiyotaka Okada
Department of Botany, Graduate School of Science, Kyoto University
An *Arabidopsis* wild-type flower has four symmetrically positioned petals whose position is fixed in the second whorl between two sepals in the first whorl. Determination of floral organ identity is understood by analyses of ABC genes, but determination of organ position in each whorl is not well understood. We isolated a mutant with defects of petal development but with normal organ identity. A single, recessive mutant, rabbit ears (*rbe*),

has the abaxial-adaxial position-dependent defects in petal development. Most of the adaxial petals are lost or transformed to filamentous, stamen-shaped, or small organs. On the other hand, defects in the abaxial petal development are fewer; therefore the remained petals look like "rabbit ears". No structural defect is observed in other floral organs and positions of the petal primordia in young floral meristem of the mutant are normal. These results indicate that RBE is involved in the growth of mainly adaxial petal primordia under control of the pre-formed abaxial-adaxial axis in the floral meristem, suggesting that the development of the adaxial and abaxial petals is differently regulated. In order to investigate the interaction between RBE and other genes involved in floral organ development, we are examining the phenotype of double mutants. We are also trying to clone the RBE gene, which is mapped at the top of chromosome 5 near the ASA1 marker.

527 COP10 MODULATES STOMATAL PATTERN IN LIGHT DEVELOPMENT IN *Arabidopsis thaliana*

Javier Torres-Contreras and Carmen Fenoll

Facultad de Ciencias del Medio Ambiente. Universidad de Castilla-La Mancha. Campus Tecnológico de la Fábrica de Armas. E-45071. Toledo. Spain

Stomatal pattern in *Arabidopsis thaliana* is strictly regulated, so that stomata never develop adjacent to a pre-formed stoma, and therefore each stoma is always surrounded by a stomata free region. The establishment of this pattern depends on the cell lineage-based mechanism associated with stomatal development, and on the acquisition of the correct cell identities during that mechanism. CONSTITUTIVE PHOTOMORPHOGENIC 10 (COP10) is a repressor of photomorphogenesis in the dark. Stomatal clusters have been described in the epidermis of cotyledons of dark-grown cop10-1 mutants, which suggests that COP10 also modulates stomatal pattern. Such stomatal clusters are also present in light-grown cop10-1 mutants. To understand the mechanism altered in cop10-1 mutants, three complementary approaches have been followed: (a) serial observation of cell divisions in the epidermis by agarose-impressions of the same cotyledon surface taken at one day intervals, (b) clonal analysis by transposon excision from the 35SGUS::Ac construct, and (c) analysis of guard mother cell and developing-guard cell identities by the expression of GUS coding-sequence from rha1 promoter, a molecular marker for such identities. The present data show that stomatal clusters arise gradually after germination, during the onset of seedling-development. They arise from altered cell identity switches in the cell lineage-based mechanism associated with stomatal development. The data show the way in which COP10 modulates stomatal pattern during light development in *Arabidopsis thaliana*.

528 CLAVATA1: Ligand binding and receptor recycling

Amy E Trotochaud and Steven E Clark

University of Michigan

CLAVATA1 (CLV1) encodes a leucine-rich-repeat receptor kinase that specifically regulates shoot and floral meristem development. In wildtype *Arabidopsis*, CLV1 is present in two distinct protein complexes. The inactive form is a 185kD complex consisting of CLV1 in a disulfide linked multimer, possibly including CLV2. The active form of CLV1 is a 450kD complex consisting of the 185kD multimer, KAPP (Kinase Associated Protein Phosphatase), ROP (a Rho GTPase), and the ligand, along with

several phosphoproteins. The 185kD disulfide linked multimer, phosphorylation and the presence of the ligand are required to form the 450kD complex. We will present data on what appears to be a central role of CLV1 phosphorylation in both ligand binding and receptor recycling. High-affinity binding of the ligand to CLV1 appears to require CLV1 kinase activity. The ligand does not bind in vivo to kinase-inactive CLV1, and ligand binding can be reversed by in vitro phosphatase treatment. In vitro phosphatase treatment disassembles the 450kD complex, leaving it only in the 185kD inactive complex. We will present evidence that this in vitro receptor recycling from the active to inactive form mirrors a similar in vivo process. Analysis of plants over- and under-expressing the protein phosphatase KAPP are consistent with the role for KAPP in recycling activated CLV1 back to the inactive form. We propose a model in which the CLV1 receptor can be activated and recycled back to the inactive state through the phosphorylation status of the kinase domain.

529 Molecular cloning and microarray analysis of the ANGUSTIFOLIA gene that regulates polar elongation of leaf cells

H. Tsukaya^{1*}, E. Nitasaka² and G.-T. Kim¹

1: Center for Integrated Bioscience and National Institute for Basic Biology, Okazaki Research Institutes, Okazaki 444-8585 Japan; 2: Graduate School of Science, Kyushu University, Hakozaki, Fukuoka 812-8581 Japan

A leaf is the fundamental unit of shoot morphogenesis. However, molecular genetic control of development of leaf shapes have remained unclear. Focusing on mechanisms that govern polarized growth of leaves in Arabidopsis, we have found that the two genes act independently to each other on the processes of polar elongation of leaf cells. The AN gene regulates width of leaves and the ROT3 gene regulates length of leaves (Tsukaya et al. 1994, Tsuge et al. 1996, Kim et al. 1998, 1999). The an mutant shows abnormal arrangement of cortical MTs in leaf cells, suggesting that the AN gene regulates polarity of cell growth via changing arrangement of cortical microtubules. Here, we report the characters of the AN gene obtained from map-based cloning of the AN gene. In addition, the function of the AN gene is discussed from data of microarray analysis. The AN gene was revealed to be a member of CtBP. CtBP is known to act as a transcriptional repressor in animal kingdom, such as human, Drosophila, mouse, Xenopus, and so on. AN is the first CtBP isolated from plants. In silico screening and real cDNA screening revealed that genomes of various dicots, including members of Dilleniidae, Rosidae and Asteridae, have AN homologs. To understand the function of the AN gene as a transcriptional regulator of the other genes, microarray analysis was carried out under the Monsanto Arabidopsis Microarray Program. As a result, it was found that the an mutant overexpresses some zinc finger genes, suggesting that the AN gene might work as a repressor of some zinc finger genes. Based on the results from analyses of the gene, molecular mechanisms related to the regulation of leaf shape will be discussed.

*This study is supported by Monsanto Arabidopsis Microarray Program.

Tsukaya et al. (1994) *Planta* 195: 309-312

Tsuge et al. (1996) *Development* 122: 1589-1600

Kim et al. (1998) *Genes & Dev.* 12: 2381-2391.

Kim et al. (1999) *Proc. Natl. Acad. Sci., USA*, 99: 9433-9437.

530 HALTED ROOT(HLR) is a key gene in the root meristem maintenance
Minako Ueda 1, 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
Meristem of primary root is established in embryogenesis. After germination, root meristem controls root growth through balanced cell proliferation and differentiation. The meristem activity is maintained during continuous root growth. Normal growth of root requires maintenance of the meristematic activity. But little is known about the maintenance machinery. We isolated an Arabidopsis mutant, halted root(hlr), which shows aberrant post-embryonic root growth. In embryo of hlr, shape and arrangement of root cells are normal. However, at 2 days after germination, root growth is halted, root tip is expanded radially, and root cap elongated abnormally. At about 7 days after germination, cell layers in the root meristem are heavily disturbed, owing to disordered cell division. Similar disturbance of meristem organization is observed in lateral roots: lateral root primordia show normal structure but lose the normal cell arrangement immediately after emergence from the parental root surface. Our data indicate that the hlr mutant undergoes normal embryogenesis, but abnormal cell divisions and disordered expansion occur in the root tip immediately after germination, suggesting that the HLR gene product is essential for maintenance of root meristem activity. We cloned the HLR gene by chromosome walking. HLR is a homologue of yeast RPT2 that is a component of 26S proteasome. It suggests that protein degradation pathway is involved in root meristem maintenance. Results for the genetic and molecular analyses will be discussed on our poster.

531 Enhancer trapping of genes expressed in the Arabidopsis embryonic shoot apex and the suspensor

Casper W. Vroemen, Kerstin Guehl, Mark Kwaaitaal and Sacco C. de Vries

. Department of Biomolecular Sciences, Laboratory of Molecular Biology, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

We are interested in Arabidopsis genes expressed early during embryo development. To find such genes, we performed an enhancer trap screen (Vroemen et al., 1998, in Cellular integration of signalling pathways in plant development, Lo Schiavo et al., eds., Springer, pp. 207-232) that employs a transposable element carrying a GUS reporter gene (Sundaresan et al., 1995, Genes & Dev. 9, 1797-1810). We are currently focussing on two lines: line WET393, that displays suspensor-specific GUS expression, and line WET368, that displays GUS expression in the early embryonic shoot apex, which subsequently gets restricted to the boundary between shoot meristem and cotyledons. Sequencing of genomic phages covering the insertion site of the WET393 enhancer trap revealed two candidate sequences for the suspensor-specific gene: a predicted single exon-gene, and a gene encoding a 14-3-3 protein. The latter class of proteins are known components of transcription factor- and other protein complexes. Screening of a developing seed-specific cDNA library yielded a full-length cDNA encoding the 14-3-3 protein, indicating that the gene is indeed expressed during early seed development. In

situ mRNA localization studies are currently carried out to determine which of the identified genes is expressed in the embryonic suspensor.

For the WET368 line, a predicted gene encoding a protein of the NAC-domain family is the most likely candidate for the observed shoot apex expression. Previously identified genes encoding NAC-domain proteins, such as the petunia NAM gene and its Arabidopsis homolog CUC2 have expression patterns very similar to that seen in the WET368 line. The NAM and CUC2 proteins are involved in organ separation and the establishment of the shoot meristem. Hence, the gene trapped by the WET368 enhancer trap may be structurally and functionally homologous to CUC2. In situ hybridization is underway to verify whether the expression pattern of the predicted gene reflects the GUS expression pattern of the WET368 enhancer trap.

532 Siamese, a regulator of the endoreplication cell cycle during trichome development

Walker, J. D. (1), Oppenheimer, D. G. (2), Joshua Concienne (1), Matt Brown (1), and Larkin, J. C. (1)

(1) Louisiana State University, (2) University of Alabama-Tuscaloosa

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 was detected due to a recessive mutation resulting in clusters of adjacent trichomes that appeared to be identical "twins" in morphology. The mutation maps north of nga158 on chromosome 5. 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.

533 POLTERGEIST functions to regulate meristem development downstream of the CLAVATA loci

Lita P. Yu, Ephraim J. Simon, Amy E. Trotochaud, and Steven E. Clark
University of Michigan, 830 N. University, Ann Arbor, MI 48109-1048

Mutations at the CLAVATA loci (CLV1, CLV2, and CLV3) result in the accumulation of undifferentiated cells at the shoot and floral meristems. We have isolated three mutant alleles of a novel locus, POLTERGEIST (POL), as suppressors of clv1, clv2 and clv3 phenotypes. Although pol mutants are nearly indistinguishable from wild-type plants,

plants homozygous for the *pol* mutation exhibit shorter pedicels and reduced shoot meristem size. *pol* mutations provided recessive, partial suppression of meristem defects in strong *clv1* and *clv3* mutants, and nearly complete suppression of weak *clv1* mutants. *pol* mutations partially suppressed *clv2* floral and pedicel defects in a dominant fashion, and almost completely suppressed *clv2* phenotypes in a recessive manner. Double mutant analysis of *pol ag* suggest that *pol* suppression of *clv* floral meristem indeterminacy is specific to the CLV loci. *pol wus* double mutants are enhanced and *wus* mutants are semi-dominant in the *pol* mutant background, indicating that POL and WUS are redundant. Based on genetic characterization, we propose that POL functions as a critical regulator of meristem development downstream of the CLV loci and redundantly with WUS.

Published data indicates that in wild-type plants the CLV1 complex is in two forms, an active 450 kD and an inactive 185 kD complex. However, in plants homozygous for the null *clv3-2* mutation, the CLV1 complex is only found in the 185 kD complex. Consistent with our hypothesis, the CLV1 complex is only found in the inactive 185 kD complex in *pol clv3-2* plants indicating that *pol* mutations do not suppress *clv3* phenotypes by altering CLV1 receptor activation.

Preliminary positional cloning indicates that the *pol-1* mutation is in an open reading frame that encodes a protein phosphatase.

534 Characterization of shoot organogenesis deficient (SOD) mutants

Qunhua Zhao and Carol Auer

Department of Plant Science, University of Connecticut, Storrs, CT 06269 USA

Shoot organogenesis is a critical process for plant improvement and propagation, but little is known about the genetic requirements for this developmental pathway. In tissue culture, differentiated explant cells go through 3 stages: acquiring competence, becoming induced (perception of plant hormone treatment), and actual shoot formation (independent of hormone treatments) Unlike arabidopsis root explants, cotyledon explants have a very short window of competence and do not normally have organ formation. Our goal was to select mutants blocked in the first 2 stages of shoot organogenesis using cotyledon explant tissue. Preliminary work with 17 ecotypes and 6 cytokinins determined that the optimal system for mutant selection was 6-d-old C24 cotyledons and a shoot induction media (SIM) containing 4.4 μ M iP and 0.5 μ M NAA. Media transfer experiments showed that competence required 2-4 days and induction required 8-10 days. The mutant screen consisted of observing excised cotyledons from over 12,000 M2 plants on SIM media and growing those lines without shoot organogenesis. Fifteen shoot-organogenesis-deficient (SOD) mutants were selected which have normal shoot apical meristems, roots and flowers. All mutants produce callus or roots in culture similar to WT. Each line was tested for cytokinin and auxin responses; some lines behaved as WT, suggesting that these mutations do not affect hormone signal transduction. For example, 6-d-old cotyledons from line 19-73 never produced shoots and only 10% of root explants produced shoots. In all SOD mutants, shoot organogenesis from root explants was not completely blocked, but the frequency decreased by more than 50% compared to WT. Information on phenotype, plant hormone responses, genetics and other experiments will be presented. Although further work is needed, the mutations

clearly change the organogenic response in differentiated cotyledon cells during the narrow window of competence and/or prior to determination for shoot formation.

535 Regulation of auxin response by SCFTIR1 and the RUB conjugation pathway
William M. Gray, J. Carlos del Pozo, and Mark Estelle
The Institute for Cellular and Molecular Biology, University of Texas, Austin, TX
78712-1095

Genetic studies in *Arabidopsis* have defined the AXR1 and TIR1 genes as factors required for auxin response. Genetic analysis suggests that AXR1 and TIR1 function in the same or overlapping pathways, and expression analysis confirms that the two genes are expressed in overlapping domains. AXR1 is related to the ubiquitin-activating enzyme (E1) and functions together with a second protein, ECR1, to activate the ubiquitin-related protein RUB1 for conjugation. We demonstrate that RUB1 is conjugated to the cullin AtCUL1.

TIR1 encodes an F-box protein with leucine-rich repeats. Studies in yeast and animal systems indicate that F-box proteins interact with SKP1 and a member of the cullin protein family to form an SCF ubiquitin-ligase complex. The F-box component of these complexes recruits specific target proteins to the SCF for ubiquitination. We find that TIR1 interacts with the Skp1p-related proteins ASK1 and ASK2 and the cullin AtCUL1 to form an SCF complex in planta. Based on these results we propose a model in which auxin response is mediated by the SCFTIR1-facilitated ubiquitination, and the subsequent degradation, of one or more negative regulators of the auxin response pathway.

Consistent with this suggestion, we find that TIR1 immunoprecipitates contain ubiquitin-ligase activity. Furthermore, overexpression of AXR1 and TIR1 promotes enhanced auxin response including increased lateral root formation and auxin-dependent gene expression. Alternatively, overexpression of a mutant derivative of AtCUL1 that cannot be modified by RUB1 confers phenotypes consistent with impaired auxin response. These results suggest that RUB1-modification of the AtCUL1 subunit of SCFTIR1 plays a crucial role in auxin response.

536 AUTOPHAGY IN PLANT-cloning of AtAPG genes and analysis of T-DNA insertion mutants-

Hideki HANAOKA¹, Takeshi NODA¹, Yumiko SHIRANO^{2,3}, Shusei SATO⁴,
Tomohiko KATO⁴, Hiroaki HAYASHI⁵, Daisuke SHIBATA^{2,4}, Satoshi TABATA⁴,
Yoshinori OHSUMI¹

¹Natl. Inst. for Basic Biol., Okazaki, Japan, ²Mitsui Plant Bio., Tsukuba, Japan, ³Univ. NJ., USA, ⁴KAZUSA DNA Res. Inst., Kisarazu, Japan, ⁵Univ. Tokyo., Tokyo, Japan
Protein degradation processes must be crucial for plant life. In plants, bulk protein degradation was induced in many situations, for example prolonged dark treatment and leaf senescence. Suspension cultured cells subjected to sucrose starvation also induce net protein degradation. Autophagy is proposed to be involved in these protein degradation processes. Autophagy, a ubiquitous process throughout eukaryotes, is an intracellular process for bulk degradation of cytoplasmic components and organelles in the lysosome/vacuole. This study aims to elucidate physiological roles of autophagy in plant. We have been studying autophagy in yeast as a model system. Under a nutrient starvation, autophagosome, a double-membrane structure surrounding a portion of

cytoplasm, is formed at the first step. Then, its outer membrane fuses to the vacuolar membrane and inner membrane structure, autophagic body, is delivered into the vacuolar lumen. Lastly, the contents of autophagic body are digested by vacuolar proteinases. Autophagy-defective yeast mutants show common phenotypes such as loss of viability during starvation and a defect in sporulation in diploid cell.

We had obtained 15 APG genes, in yeast, whose gene products were revealed to be essential and directly involved in autophagy. Seven cDNAs homologous to yeast APG genes were cloned from *Arabidopsis thaliana* (AtAPG genes). To obtain autophagy-defective plant mutants, we searched for the *A. thaliana* lines containing T-DNA insertion within those genes and obtained 2 candidates, *atapg9-1* and *atapg4b-1*. AtAPG9 is predicted to be a 99 kDa membrane spanning protein, and AtAPG4b is predicted to be a 52 kDa hydrophilic protein. Though T-DNAs were inserted within an intron, full-length cDNA of AtAPG9 or AtAPG4b was not detected by RT-PCR analysis in the corresponding mutant lines homozygous for T-DNA insertion. Both mutant lines could produce seeds and exhibited normal development under nutrient rich condition. The phenotypes of mutant lines under various nutrient starvation conditions will be reported.

537 Control of flower development and meiosis by ASK1, a homolog of a key regulator of proteolysis in yeast

MA, Hong, Dazhong Zhao, Yi Hu, and Ming Yang

Department of Biology and Life Sciences Consortium, Penn State University, PA 16802 USA

The yeast SKP1 gene is essential for the mitotic cell cycle and encodes a regulator of proteolysis. SKP1 interacts with an F-box protein and cullin to form the SCF ubiquitin ligase complex, which targets specific proteins to the ubiquitin-dependent degradation pathway. *Arabidopsis* has more than a dozen SKP1 homologs that have been named ASK genes. We have identified a mutation in the ASK1 gene among our Ds insertion collection and the *ask1-1* mutant exhibit developmental abnormalities during vegetative and floral development [1,2]. The *ask1-1* mutant rosette plant is smaller than normal and has reduced floral stem length, both as a result of reduced cell number. In addition, the mutant flower has reduced petal size and shortened filament length in the stamens. These phenotypes are consistent with a defect in the mitotic cell cycle. The *ask1-1* mutant also has a weak effect on floral organ identity in whorls 2 and 3, and genetically interacts with the UFO gene, which also affects whorls 2 and 3. UFO is an F-box protein and its physical interaction with ASK1 was shown in a yeast 2-hybrid assay by W. Crosby and G. Haughn and colleagues [3]. Our recent studies suggest that, similar to UFO [4], ASK1 interacts with LFY to activate the expression of AP3; therefore, ASK1 and UFO may target a negative regulator of LFY function or AP3 expression. The *ask1-1* mutant is also male sterile and exhibits a severe defect in homologous chromosome separation at anaphase I [1]. This is consistent with the idea that in the mutant the homologs can pair but the pairing structures remain unresolved at anaphase I, preventing proper separation and causing mis-segregation of homologues. Therefore, ASK1 may be required for the degradation of a regulator of the chromosome pairing apparatus.

We thank Bill Crosby, George Haughn, and Detlef Weigel for sharing unpublished results and materials.

This work is supported by grants from the US NSF and USDA and by the Penn State University Life Sciences Consortium.

1. Yang et al. 1999. PNAS, USA 96: 11416-11421.
2. Zhao et al. 1999. Dev. Genet. 25: 209-223.
3. Samach et al. 1999. Plant J. 20: 433-445.
4. Lee et al. 1997. Curr. Biol. 7: 95-104.

538 SUMO pathway: molecular and genetic analyses

Jasmina Kurepa and Richard D. Vierstra

Cellular and Molecular Biology Program and Department of Horticulture, University of Wisconsin-Madison, Madison, WI 53706, USA

Post-translational modification of proteins is an important way of modulating cellular functions. Peptide tags are a group of post-translational modifiers that recently emerged as a more numerous and divergent family than previously thought. The first polypeptide tag identified was ubiquitin, which when bound to a protein marks it for degradation by the 26S proteasome. Recently, other tags such as RUBs (related to ubiquitin), APG12, URMIs (ubiquitin related modifier) and SUMOs (small ubiquitin-like modifier) have been detected in plants. The Arabidopsis SUMO family has 8 members that can be grouped into three subfamilies which may modify a different subsets of target proteins. Like other peptide tags, SUMOs are covalently linked to other proteins in an ATP-dependant enzymatic cascade. We have identified enzymes of the SUMO conjugation pathway in Arabidopsis: the heterodimeric SUMO activation enzyme (SCE1/SCE2) encoded by three genes (AT-SAE1-1, AT-SAE1-2 and AT-SAE2) and the SUMO conjugation enzyme (SCE1) encoded by a single gene AT-SCE1. Studies in yeast and animals have implicated the SUMO pathway in nucleocytoplasmatic and intranuclear targeting as well as in the stress response. To analyze the function of this modifier pathway in plants, we have isolated T-DNA insertion mutants in SUMO pathway genes and generated plants with modified levels of SUMO or SCE1. In additions, plants expressing SUMO1 and SUMO2 with a N-terminal hexahistidine tag are being used to identify SUMO targets. For this purpose, proteins isolated by Ni-NTA chromatography will be analyzed by mass spectrometry. The expression pattern of SUMO pathway genes as well as developmental alterations associated with SUMO knockout mutants will be presented.

539 A role of Arabidopsis pleiotropic COP/DET/FUS proteins in light-regulated HY5 degradation

Mark T. Osterlund, Christian S. Hardtke, Ning Wei, and Xing Wang Deng

Department of Molecular, Cellular and Developmental Biology Yale University, PO Box 208104 165 Prospect Street, OML 301 New Haven, CT 06520-8104, USA

Arabidopsis seedlings display contrasting development patterns depending on the ambient light environment. Seedlings grown in the light develop photomorphogenically, characterized by short hypocotyls and expanded green cotyledons. In contrast, seedlings grown in darkness become etiolated with elongated hypocotyls and closed cotyledons on an apical hook. Light signals, perceived by multiple photoreceptors and transduced to downstream regulators, dictate the extent of photomorphogenic development in a quantitative manner. Two key downstream components, COP1 and HY5, act antagonistically in regulating seedling development. HY5 is a bZIP transcription factor

that directly binds to the promoters of light inducible genes, promoting their expression and photomorphogenic development. COP1 is a RING-finger protein with WD-40 repeats whose nuclear abundance is negatively regulated by light. It has been demonstrated that in the nucleus, COP1 directly interacts with HY5 to negatively regulate its activity. Here we present evidence that the abundance of HY5 is directly correlated with the extent of photomorphogenic development, and that the COP1-HY5 interaction may specifically target HY5 for proteasome mediated degradation in the nucleus. All other pleiotropic COP/DET/FUS proteins are essential for this light-regulated HY5 instability.

540 Characterization of a multigene family encoding RING-H2 zinc fingers in Arabidopsis

Socorro Parra, Jaime Bravo, Laura Aguilar and Plinio Guzman

Genetic Engineering Department. Cinvestav-IPN, Unidad Irapuato. Irapuato, Gto, Mexico

The RING finger motif is ubiquitously found in eukaryotes in a wide range of proteins with apparently unrelated function. Although few RING finger genes have been characterized in plants, more than 50 genes encoding the RING finger motif or the RING-H2 variation can be predicted in Arabidopsis from the analysis of the sequence of chromosome 2 and 4. New observations in yeast and mammals discussing the essential role of the RING finger motif on the mechanism of protein proteolysis by ubiquitination raises the question of whether in plants this type of motif can also have a role in proteolysis. The ubiquitin-dependent pathway is a highly conserved process among eukaryotes, including plants, that involves at least three classes of proteins (E1, E2 and E3). Recent observations indicate that the RING finger proteins are part of several E3s, a component responsible for selecting the substrate to be ubiquitinated and degraded. This finding suggests that a common theme can be inferred for the RING finger. If each of the numerous RING finger proteins predicted in plants exhibit specificity for a substrate, ubiquitin-dependent degradation will influence a vast array of processes. We are continuing the characterization of a family of RING-H2 proteins, named ATL. This family share various structural features: an almost identical RING-H2 domain at the central region of the polypeptide, a putative membrane-spanning domain at the N-terminus, followed by a region rich in basic amino acid residues and a region showing primary sequence conservation; about 38 polypeptides sharing such features can be predicted from the analysis of the sequence of chromosome 2 and 4. We are currently establishing the pattern of expression of various members of this family in transgenic Arabidopsis plants containing promoter fusions to reporter genes. We are also using the yeast two-hybrid system to search for proteins that may interact with ATLs and we are directly searching for interaction of ATLs with E2 conjugating proteins. Advances of this research will be presented. This work is supported by a grant from CONACyT, México.

541 COP9 signalsome-a developmental regulator involved in ubiquitin/proteasome pathway

Zhaohua Peng, Ning Wei, Xing-Wang Deng

Dept. of MCDB, Yale University

COP9 signalsome was originally isolated as a photomorphogenic repressor mediating light regulated development in Arabidopsis. It is a protein complex composed of 8 subunits and conserved from human to plants. The 8 subunits of the COP9 signalsome share homology with the 8 subunits of proteasome lid subcomplex in a one to one correlation. We demonstrated that mutations of COP9 signalsome accumulated large amounts of ubiquitinated proteins and a small portion of COP9 signalsome was associated with proteasome by both gel filtration and co-immunoprecipitation analyses. Further, it was found that mutation in COP9 complex only affected some specific protein degradation, for example, PHYA degradation was not affected in the mutants but HY5 degradation was affected. The role of COP9 signalsome in development was demonstrated in both antisense and sense co-suppression transgenic plant studies of the S6 subunit. These transgenic plants displayed diverse homeotic mutation phenotypes in flower development, such as sepal transformed to carpel, missing sepals, petals, stamens and carpels, Cauline leaf transformed to carpel and so on. Even in a single transgenic line, 20 different leaf types with dramatic morphological changes have been found. Further, phenotypes in root, cotyledon and silique development have also been observed. A model of COP9 signalsome action is proposed.

542 Characterization of the ASK subunit in SCF ubiquitin ligases

Eddy Risseuw, Enwu Liu, Travis Banks, Tim Daskalchuk, Bill Gray¹, Mark Estelle¹, Jennifer Klenz², George W. Haughn², Ming Yang³, Hong Ma³ and William L. Crosby
Plant Biotechnology Institute - NRC, 110 Gymnasium Place, Saskatoon SK S7N 0W9, Canada. 1)University of Texas, Austin, TX 78712, USA. 2)University of British Columbia, Vancouver BC V6S 1A4. 3)Pennsylvania State University, University Park PA 16802, USA

Targeted degradation of regulatory proteins mediated by the ubiquitin pathway, is an important mechanism in numerous cellular and developmental processes in all eukariotes. Recently, a novel class of ubiquitin ligases (E3) was discovered that consists of the subunits Skp1, Cdc53/Cullin and an F-box protein (SCF). The F-box proteins are thought to be receptors for specific target proteins. Skp1 mediates the interaction between the F-box and Cdc53/Cullin which recruits the ubiquitin conjugating enzyme E2. Upon binding to the SCF, the target protein becomes polyubiquitinated and rapidly degraded by the 26S proteasome. The UNUSUAL FLORAL ORGANS (UFO) gene from Arabidopsis encodes an F-box protein and interacts with the Arabidopsis SKP1-like proteins ASK1 and ASK2. The same ASK proteins also interact with other F-box proteins like TIR1 involved in auxin signal transduction. The ask1-1 mutant has a floral phenotype that reveals some characteristics of ufo and is like tir1-1, resistant to low auxin levels. Both ASK1 and ASK2 complement the ask1-1 mutant, however ASK2 is only capable when expressed by the 35S promoter but not by the ASK1 promoter. Over expression of ASK1 and ASK2 result in a similar phenotype with tall leaves, early flowering and increased rate of flower formation, suggesting an increased meristematic activity. In addition these lines show defects in the inflorescence architecture and are resistant to auxin, which is typical for ufo and tir1-1, respectively. The role of ASK in different pathways will be discussed.

543 Functional analysis of the 26S Proteasome subunits from Arabidopsis thaliana

Jan Smalle¹, Jasmina Kurepa¹, Elena Babyichuk², Sergei Kushnir² and Richard D. Vierstra¹

¹Cellular and Molecular Biology Program and the Department of Horticulture, University of Wisconsin-Madison, WI 53706, USA., ²Vlaams Interuniversitair Instituut voor Biotechnologie (VIB), Universiteit Gent, B-9000 Gent, Belgium

Cellular processes that depend on rapid changes in protein activities often involve the specific degradation of targets by the ubiquitin/26S proteasome pathway. Ubiquitin dependent degradation essentially consists out of two parts. First, a target protein becomes multiubiquitinated through the sequential action of a set of activating, conjugating and ligating enzymes. Subsequently, proteins become targets for degradation by the 26S proteasome, a large multisubunit complex consisting out of two subparticles: the 20S proteasome and the 19S regulator. Whereas the proteolytic activity entirely resides within the 20S part, the 19S is thought to assist in the recognition of target proteins thus provides substrate specificity. Studies in yeasts, animals and plant cell cultures have implicated the proteasome in a number of cellular processes, including the regulation of cell division, metabolism, development, apoptosis, and the immune response. To define the function of the 26S proteasome in plant homeostasis and development, we are characterizing a set of mutants carrying T-DNA insertions in various 19S and 20S subunit genes. As expected, disruption of a single-copy 20S subunit gene causes embryonic lethality. However, mutations affecting different 19S subunits resulted in distinct developmental phenotypes. Our data suggests a role for the 19S subcomplex in controlling meristem function, leaf senescence and the response to cytokinins, probably by controlling the specific degradation of regulatory factors.

544 The Function of AtGRR1 in *Arabidopsis thaliana*

WenYan Xiao and Jyan-Chyun Jang

Dept. of Horticulture and Crop Science, Ohio State University, Columbus, Ohio 43210

GRR1 plays a central role in the glucose signal transduction pathway in the yeast *Saccharomyces cerevisiae*. Mutations in GRR1 result in a pleiotropic phenotype, including defective glucose signaling, reduced transport of divalent cations and amino acids, and elongated cell morphology. It has been demonstrated recently that GRR1 is an F-box protein which is a component of E3 ubiquitin protein ligase termed SCF complex. SCFGRR1 is required for the degradation of G1 cyclins in cell cycle. To understand whether plant sugar signaling involves a similar component such as the yeast GRR1, we use reverse genetic approaches. By database search, we have identified a number of novel *Arabidopsis* F-box proteins showing high homology to the yeast GRR1. To test whether an ortholog of the yeast GRR1 exists in *Arabidopsis*, we perform yeast complementation using two *Arabidopsis* cDNA (AtGRR1.1 and AtGRR1.2) with the highest sequence similarity to the yeast GRR1, as well as using an *Arabidopsis* cDNA library. The results will be presented. We have also explored the biological functions of one of these two GRR1 homologs in *Arabidopsis* by its misexpression in transgenic plants. Although no altered sugar response has been observed thus far, the transgenic plants have shown altered sensitivity to divalent cations, such as cobalt, consistent with the phenotype of the yeast *grr1* mutants. Further characterization of these transgenic plants and the search for Knockout alleles and GRR1-interacting partners are in progress.

545 Genetic Engineering of a Self-destructing Winter Cover Crop

Marisha Stanislaus and Chi-lien Cheng

Department of Biological Sciences, University of Iowa, Iowa City, IA 52242

The use of winter cover crops is beneficial to agriculture. Cover crops are planted in the late fall and serve to protect the soil from erosion and replenish it with essential nutrients such as nitrates. However, their removal in late Spring requires the use of tillage and herbicides. We have aimed to design a cover crop that self destructs in response to an environmental cue, thereby eliminating the use of herbicides and tillage. The onset of summer brings with it elevated temperatures. Using this as the environmental cue, a self-destructive cassette was designed. The promoter of the gene encoding the heat shock protein, hsp 81-1, of *Arabidopsis thaliana*, was used to drive the expression of the ribonuclease, Barnase, of *Bacillus amyloquelaciens*. Since Barnase is extremely toxic to the cell, it was necessary to co-express its inhibitor, Barstar, which was driven by the CaMV 35S promoter and linked in tandem to Barnase. Four versions of Barnase were used, a wild-type version and three mutant versions, namely attenuated, missense and nonsense. In the initial study, tobacco was used as a model system. Our results showed that the attenuated version of Barnase was the most effective in causing heat shock regulated plant death. Barnase gene expression was 3-4 x higher in heat shock treated plants compared to untreated plants. Heat shock treatment was confirmed by induction, of the HSP 70 gene. The T1 transgenics had multiple T-DNA insertions that were transmitted to the next generation. Barnase gene expression was seen to vary among the T2 progeny, possibly due to varying degrees of gene silencing effects.

546 *Arabidopsis* rocket science

Bratislav Stankovic

University of Wisconsin - Madison

The global impact of plant biology extends beyond the Earth's boundaries. Plants are being evaluated in the NASA's Advanced Life Support program as a key tool for regeneration of the atmosphere, water purification, and food production during long-duration space missions. With the prospect of available microgravity laboratory facilities for long-term studies on the International Space Station (ISS), plants are being exploited for space commercialization. The Wisconsin Center for Space Automation and Robotics (WCSAR) is a NASA-cosponsored Commercial Space Center whose mission is to support industry in the development and commercialization of new products and technologies derived from plant research conducted in microgravity. WCSAR has developed several patented, controlled environment technologies, which provided the basis for design of microgravity-based plant research facilities, including Astroculture (Space Shuttle-based), Advanced Astroculture (ISS-based), and Commercial Plant Biotechnology Facility (ISS-based). The Wisconsin Center for Space Automation and Robotics has conducted nine commercial experiments on board the Space Shuttle and the MIR Space Station. These microgravity experiments have ranged from studies on plant growth, through isolation and analysis of novel secondary compounds, to plant transformation. WCSAR is positioning itself for long-term microgravity-based *Arabidopsis* research on the ISS that will yield both fundamental understanding of the effects of gravity/microgravity on plant growth and metabolism, and applied data whose objective is space product development.