75 Integrating Membrane Transport with Male Gametophyte Development and Function through Transcriptomics

Kevin Bock[1], David Honys[4], John Ward[5], Senthilkumar Padmanaban[1], Eric Nawrocki[1], Kendal Hirschi[2], David Twell[3], Heven Sze[1]

[1]University of Maryland, College Park, MD, [2]Baylor College of Medicine, Houston, USA , [3]University of Leicester, Leicester, U K , [4]Institute of Experimental Botany ASCR, Prague, Czech Republic, [5]University of Minnesota, St. Paul, MN, USA

Male fertility depends on the proper development of the male gametophyte, successful pollen germination, tube growth and delivery of the sperm cells to the ovule. Previous studies have shown that nutrients like boron, and ion gradients or currents of Ca^{2+}, H^+, and K^+ are critical for pollen tube growth. However, the molecular identities of transporters mediating these fluxes are mostly unknown. As a first step to integrate transport with pollen development and function, a genome-wide analysis of transporter genes expressed in the male gametophyte at four developmental stages was conducted. About 1269 genes encoding classified transporters were collected from the Arabidopsis thaliana genome. Of 757 transporter genes expressed in pollen, 16% or 124 genes, including *AHA6*, *CNGC18*, *TIP1.3* and *CHX08*, are specifically or preferentially expressed relative to sporophytic tissues. Some genes are highly expressed in microspores and bicellular pollen (*COPT3, STP2, OPT9*); while others are activated only in tricellular or mature pollen (*STP11, LHT7*). Analyses of entire gene families showed that a subset of genes, including those expressed in sporophytic tissues, were developmentally-regulated during pollen maturation. Early and late expression patterns revealed by transcriptome analysis are supported by promoter::GUS analyses of *CHX* genes and by other methods. Recent genetic studies based on a few transporters, including plasma membrane H^+ pump AHA3, Ca^{2+} pump ACA9, and K^+ channel SPIK, further support the expression patterns and the inferred functions revealed by our analyses. Thus, revealing the distinct expression patterns of specific transporters and unknown polytopic proteins during microgametogenesis provides new insights for strategic mutant analyses necessary to integrate the roles of transporters and potential receptors with male gametophyte development.

1. Supported in part by NSF Arabidopsis 2010 grant #0209792 to JMW, #020977 to KDH, #IBN0209788 to HS and by Department of Energy grant DEFG0295ER20200 to HS. DT and DH gratefully acknowledge support from the Royal Society Joint Project Grant 2004/R3-EU. DH was supported through a Grant Agency of the ASCR grant KJB6038409. DT gratefully acknowledges support from BBSRC and the GARNet transcriptome centre at NASC for performing pollen microarray hybridizations

76 To identify the biotinylation of histones in Arabidopsis

xi chen

iowa state university

Histones initially were regarded as only structural components but now are recognized for regulating gene expression by changing the dynamic equilibrium of chromatin. The amino termini of histones (histone tails) protrude out of the nucleosomal surface and are accessible to be modified. Histones, especially residues of the amino termini of histones H3 and H4 and the amino and carboxyl termini of histones H2A, H2B and H1, are susceptible to variety of post-translational modifications: phosphorylation, acetylation, methylation and so on. In 2001, Steven J *etl* found a novel modification, biotinylation of histones and biotinylation increases early in the cell cycle. We consider whether this kind of modification happen in the plants, especially in Arabidopsis. Because of its small genome, Arabidopsis serves as a powerful system for understanding the role of various histone modifications in a complex organism. Therefore, we hope that our research can enrich the known knowledge of histone modification, even histone code.

77 Alterations in Sphingolipid Hydroxylation Have Profound Effects on Plant Growth and Sphingolipid Composition

Ming Chen[1], Jonathan Markham[1], Charles Dietrich[2], Jan Jaworski[1], Edgar Cahoon[2]

[1]Donald Danforth Plant Science Center, 975 N. Warson Rd., St. Louis, MO 63132, [2]USDA-ARS Plant Genetics Research Unit, Donald Danforth Plant Science Center

Sphingolipids are major structural components of the plasma membrane and tonoplast of plant cells and are enriched, along with sterols, in detergent resistant membrane fractions or lipid rafts prepared from plasma membrane. Lipid rafts are also enriched in GPI-anchored proteins, which have been linked with a number of cell surface-related functions, including cell wall synthesis and signal transduction. One goal of our research is to understand the effects of sphingolipid composition on plant growth and development. In this study, Arabidopsis mutants with defects in the C-4 hydroxylation of sphingolipid long-chain bases (LCBs) were characterized. The C-4 LCB hydroxylase catalyzes the conversion of dihydroxy LCBs to the trihydroxy form. Typically, ~90% of the sphingolipids in Arabidopsis leaves contain tri-hydroxy LCBs. Two genes for the C-4 LCB hydroxylase occur in Arabidopsis. T-DNA mutants for either gene displayed partial reductions in the tri-hydroxy LCB content of sphingolipids. These mutants, however, had no observable growth phenotype. By contrast, double mutants of the two hydroxylase genes, which completely lacked trihydroxy LCBs, were severely dwarfed and did not bolt. By use of RNAi, reductions in the growth of plants were found to be closely correlated with the trihydroxy LCB content. In addition, global analysis of sphingolipids in the C-4 hydroxylase mutants revealed large alterations in the composition of all sphingolipid classes. These results demonstrate that relatively small changes in the structure of sphingolipids (e.g. the loss of a single hydroxyl group) can have profound effects on the growth and development of Arabidopsis.

This work was supported by a National Science Foundation Arabidopsis 2010 grant (MCB-0313466) "Collaborative Research: The Synthesis and Function of Arabidopsis thaliana Sphingolipids."

78 Authentic Investigations as Pedagogical Tool for Learning Scientific Inquiry

David Lally, Julia Grady, Dayna Wilhelm, Marshall Swafford, Erin Dolan

Virginia Tech

We hypothesize that, by engaging in authentic investigation (i.e., experiments that have no known outcomes and that are of interest to the broader scientific community), students can learn the process of scientific inquiry, especially determining how data constitute evidence, generating alternative explanations, and making connections between evidence and explanations. We anticipate that authentic investigations can serve as a sustainable mechanism for partnership among students, teachers, and scientists because they are bi-directional in nature and execution and they consider the needs and resources of all partners. For example, students can offer many pairs of hands, teachers can offer expertise in communicating with non-technical audiences, and scientists can offer experimental resources and know-how. An inquiry-based biotechnology education program, the Partnership for Research and Education in Plants (PREP; www.prep.biotech.vt.edu), serves as a framework for determining the outcomes and impacts of authentic investigation and partnership. PREP brings together high school teachers and research scientists to guide high school students in characterizing genes in Arabidopsis thaliana. Scientists provide wild-type and mutant (T-DNA insertion line) seeds and experimental know-how to students, and students design and conduct experiments to examine the effects of abiotic stressors (e.g., drought, salinity, soil pH, etc.) on wild-type vs. mutant plants, thereby helping to determine the function of each altered gene. We are analyzing student lab reports to determine how students use their research questions to guide their experiment design, data collection, and data analysis, as well as how students explain the implications of their findings.

79 PREP: Partnership for Research and Education in Plants

Eric Brooks[1, 2], Erin Dolan[3], David Lally[3], Carol Robertson[4, 5], Frans Tax[1], John Walker[4], Margaret Wilch[1, 6]

[1]University of Arizona, [2]Buena High School, [3]Virginia Tech, [4]University of Missouri, [5]Fulton High School, [6]Tucson High Magnet School

Everyone can think of a time in their own science education when they conducted an "experiment" with a predictable outcome, begging the question, "Why are we doing this?" When such activities don't yield the anticipated result, the focus of students' scientific discussions becomes, "What went wrong?" rather than, "How do these data address our scientific question?" or, "What alternative explanations could account for our findings?" Although demonstrations, as well as lab activities with expected results, are valuable teaching tools, students who have only these sorts of science learning experiences miss an opportunity to experience the excitement of authentic investigation and understand the empirical nature of science. Through the Partnership for Research & Education in Plants (PREP, www.prep.biotech.vt.edu; funded by a Science Education Partnership Award from the National Institutes of Health National Center for Research Resources), students can ask and answer their own questions to yield unknown and unanticipated results in the context of an ongoing effort to characterize gene function in Arabidopsis thaliana.

PREP brings together high school teachers and research scientists to guide high school students in designing and conducting authentic investigations (i.e., experiments that have no known outcomes and that are of interest to the broader scientific community). Scientists provide wild-type and mutant (T-DNA insertion line) seeds and experimental know-how to students, and students design experiments to examine the effects of abiotic stressors (e.g., drought, salinity, soil pH, etc.) on wild-type vs. mutant plants, thereby helping to determine the function of each altered gene. In addition, a select group of PREP teachers are serving as Fellows (funded by the National Science Foundation's 2010 Project). Through summer research experiences and ongoing academic-year communication, the Fellows collaborate with PREP scientists and program personnel to integrate authentic investigation into their science teaching, act as motivators and mentors for their colleagues, serve as advisors for the improvement and expansion of PREP, and develop components of a genomics teaching and learning toolkit. PREP and the PREP Fellows program serve as contexts for investigating the following hypotheses: (1) By engaging in authentic investigation, students can learn the process of scientific inquiry, especially determining how data constitute evidence, generating alternative explanations, and making connections between evidence and explanations, and (2) Partnerships between research scientists and K-12 science teachers have the potential to integrate authentic investigation into classroom learning and enhance public understanding of science.

80 The genomic pattern of linkage disequilibrium in Arabidopsis thaliana

Tina Hu[1], Sung Kim[1], Vincent Plagnol[1], Chris Toomajian[1], Richard Clark[2], Clare Lister[3], Caroline Dean[3], Joseph Ecker[4], Detlef Weigel[2], Magnus Nordborg[1]

[1]Department of Biological Sciences, University of Southern California, Los Angeles, California, USA, [2]Department of Molecular Biology, Max Planck Institute for Developmental Biology, Tubingen, Germany, [3]Cell and Developmental Biology, John Innes Centre, Norwich, UK, [4]Department of Plant Biology, The Salk Institute for Biological Studies, La Jolla, California, USA

We describe the pattern of linkage disequilibrium in 20 inbred accessions of Arabidopsis thaliana, using a dense set of over 250,000 non-singleton single nucleotide polymorphisms (SNPs) generated using Perlegen whole-genome re-sequencing oligonucleotide tiling arrays. Linkage disequilibrium decays rapidly (within 10kb) in general, but varies greatly across the genome and is strongly correlated with direct estimates of recombination. On a finer scale, we find that linkage disequilibrium appears to be higher within rather than between genes, indicating that recombination may be suppressed in genes. However, the decay of linkage disequilibrium is also slower for nonsynonymous as compared to synonymous polymorphisms, demonstrating the effect of selection.

81 Insights into the Plant Polyadenylation Apparatus

Arthur Hunt[3], Balasubrahmanyam Addepalli[3], Srinivasa Rao[3], Kevin Forbes[3], Kimberly Delaney[3], Ruqiang Xu[1], Quinn Li[1], Kil-Young Yun[2], Deane Falcone[2]

[1]Miami University, Oxford, OH, [2]University of Massachusetts, Lowell, MA, [3]University of Kentucky, Lexington, KY

The Arabidopsis genome possesses genes capable of encoding at least fifteen polyadenylation factor subunits; eight of these are encoded by single genes, while the rest are specified by gene families with two-four members. To gain a better understanding of these genes, a comprehensive protein-protein interaction map of the plant polyadenylation apparatus has been assembled. More than 50 different pairwise interactions may be inferred from the combination of yeast two-hybrid, copurification, and phage display studies. The results reveal a complicated array of interactions amongst these proteins, and suggest that different members of the various gene families may encode subunits with different properties.

The phage display studies involve a screening of a random combinatorial library for short peptides that interact with purified recombinant polyadenylation factor subunits. Comparison of peptides so identified with the Arabidopsis proteome has resulted in the identification of a number of proteins, apart from identifiable polyadenylation factor subunits, that may interact with the polyadenylation apparatus. Several of these interactions have been tested using two-hybrid and in vitro assays, and a number of novel interacting partners identified. These partners link mRNA 3' end formation with processes as diverse as transcription initiation, protein turnover, and lipid signal transduction.

One of the subunits that are encoded by single genes is the counterpart of the 30kD subunit of CPSF (AtCPSF30). Interestingly, this gene is not essential, as T-DNA insertions that interrupt the gene and eliminate the production of mRNA and protein are viable. The non-essential nature of AtCPSF30 is interesting, as the corresponding gene in yeast is essential. AtCPSF30 interacts with calmodulin, and its RNA-binding activity is inhibited by calmodulin in a calcium-dependent manner. These observations raise the possibility that AtCPSF30 may contribute to cellular signaling mediated by calmodulin.

This work is supported by NSF Arabidopsis 2010 Grant MCB-0313472.

82 The *LATERAL ORGAN BOUNDARIES (LOB)* Transcription Factor Binds a Cis-Element In Vitro

Aman Husbands, Harley Smith, Patricia Springer

University of California - Riverside

LATERAL ORGAN BOUNDARIES (LOB) is the founder of the LOB DOMAIN (LBD) gene family, a novel plant-specific family of putative transcription factors. LOB is expressed in organ boundaries and its biological function remains unclear. LBD proteins are characterized by the presence of the ~100 amino acid LOB domain, which is highly conserved in all members of the family. The LOB domain contains two conserved blocks, one of which resembles a Zn^{2+} finger domain, while the other has the hallmarks of a Leu-zipper. Thus, this family has the potential to bind DNA and to interact with other proteins. We tested the ability of the LOB Domain to bind DNA using the Selection and Amplification Binding (SAAB) assay. We identified a specific DNA sequence that is bound by LOB. Electrophoretic Mobility Shift Assays (EMSAs) were performed to confirm that the full-length LOB protein binds to this sequence *in vitro*. Competition experiments confirm the binding to be specific. Another LBD protein was also shown to bind the same DNA motif, supporting the hypothesis that this family of transcription factors binds the same cis-element.

83 Characterization of Orthologous Subtilases in Arabidopsis and Tomato

Franziska Huttenlocher, Anna Cedzich, Katrin Ullrich, Annick Stintzi, Andreas Schaller

University of Hohenheim, Institute of Plant Physiology and Biotechnology, 70599 Stuttgart, Germany

Subtilases or subtilisin-like serine proteinases are encoded by large gene families in higher plants. In Arabidopsis thaliana, 56 subtilase genes have been annotated. Within the scope of the AFGN program and in collaboration with four European and partners, we are aiming at the functional characterization of the Arabidopsis subtilase family using T-DNA insertion mutants, detailed expression analysis, and computational approaches (Rautengarten et al., 2005). However, loss-of-function analysis revealed obvious phenotypical differences for only two of the mutants, i.e. sdd1 and ale1. The vast majority of the T-DNA-insertion lines did not exhibit any defects under standard growth conditions (cf. poster by Knappenberger et al.). To gain further insight into the function of the gene family, we extend our studies to tomato, comparing the organization of the subtilase gene family, the expression of orthologous genes and the properties of the enzymes.

Phylogenetic analysis suggests the tomato genes for subtilases LeSBT1, 2, and 3 to be orthologous to At5g67360, At5g51170 and At5g67090 , respectively. Functional equivalence of the three pairs of genes is supported by similar expression patterns: promoter::GUS analysis indicated expression for LeSBT1 and At5g67360 in the vasculature, in sepal and petal abscission zones and in the style. LeSBT2 and At5g51170 were found to be expressed similarly in guard cells, while the promoter activity of LeSBT3 and At5g67090 was detected in the vasculature as well as in roots, particularly at the root tip and the sites where lateral roots protrude. For the analysis of the biochemical properties, LeSBT3 was overexpressed in a plant cell suspension culture and purified to homogeneity f rom culture supernatants. Using synthetic oligopeptides as substrates, MALDI-TOF MS analysis of the cleavage products indicated a preference of recombinant LeSBT3 for glutamine in the P1 position of its substrates. A detailed kinetic analysis of the LeSBT3 enzyme was performed using a fluorigenic peptide substrate derived from the preliminary analysis of substrate specificity. How the properties of recombinant LeSBT3 compare to those of the corresponding Arabidopsis subtilase remains to be seen.

Ref.: Rautengarten, C., Steinhauser, D., Büssis, D., Stintzi, A., Schaller, A., Kopka, J., Altmann, T. (2005): Inferring hypotheses on functional relationships of genes: Analysis of the Arabidopsis thaliana subtilase gene family. PLoS Comput. Biol. 1(4): e40

84 Analysis of the Arabidopsis thaliana Subtilisin-Like Serine Protease Gene Family

Mathias Knappenberger, Annick Stintzi, Andreas Schaller

Institute of Plant Physiology and Biotechnology - University Hohenheim - 70593 Stuttgart - Germany

Subtilases or subtilisin-like serine proteinases are the closest homologs in plants of mammalian proprotein or prohormone convertases. In *Arabidopsis thaliana*, they are encoded by a gene family of 56 members.

Thus far, a specific function has been assigned to just two of them: SDD1 specifies stomatal density and distribution and ALE1 is required for cuticle formation and epidermal differentiation during embryo development. In collaboration with four European and partners and supported by the AFGN program (AL 387/5 SCHA 591/3), we seek to understand the function of additional members of this large gene family. Our functional studies involve the characterization of loss-of-function T-DNA insertional mutants, and expression analyses for the entire gene family.

To date, for plants grown under standard conditions, no phenotypic changes were observed within the 90 T-DNA insertion lines corresponding to 49 individual subtilase genes.

Spatial and temporal subtilases gene expression was specified for 33 members of the family by analyzing β-glucuronidase staining patterns in transformed Arabidopsis plants. The majority of the transgenics displayed complex yet distinct patterns of reporter gene expression, SBT3.14 being the only one so far that is specifically expressed in the inner integument of the developing Arabidopsis seed coat. For this line, GUS and GFP signals were first detected at the micropylar end of ovules at stage 12 *i.e.* before floral bud opening. The expression persisted until stage 17, at which it also extended to the chalazal area.

While the expression pattern of SBT3.14 suggests a role in seed development, no morphological differences could be identified between wild-type and mutant ovules or seeds. In order to test whether the loss of SBT3.14 function results in a “molecular” phenotype, Microarray experiments were performed using RNA extracted from stage 12-17 wild-type and mutant gynoecium. Progress on different aspects of the project will be presented.

85 Systems Biology at NASC

Sean May
NASC

NASC provides a detailed and usable interface for browsing genomic information via the Ensembl genome browser. Genome assembly and annotation is provided by both MIPS and TAIR (version 6) models together with alignments performed at NASC of the latest sequence data such as DNA and EST data. Features such as Affymetrix probes are linked to external sources including our large database of Affymetrix expression data. We aim to provide the full range of Ensembl features, including data download and access to the Ensembl API.

Affymetix data and services : affymetrix.arabidopsis.info/

Since February 2002, the NASC has provided a comprehensive affymetrix service. Since spring 2005 we have also been accepting data donation. All of the data we produce and receive are made available to the public. This is done through our database NASCarrays, at http://affymetrix.arabidopsis.info. On our website is a full MIAME- supportive description of the experiments, and the ability to download these experiments in a convenient form. Also on our website are data/mining tools for searching our entire database. Our data is submitted to ArrayExpress, the EBIs central repository for microarray data. This is utilised by Mapman and GenevestigATor for community use.

Ontologies : Arabidopsis.info/bioinformatics/Ontology_details.html

NASC are also keen to promote the use of ontologies and standards to enhance the annotation of data stored in our germplasm catalogue. One of the first steps has been to use the current Plant Ontology (PO) with Phenotype and Trait Ontology (PATO) to curate germplasm phenotype data using Entity Attribute Value (EAV) descriptions.

86 NASC Germplasm Resources: http://arabidopsis.info

Sean May
NASC

The Nottingham Arabidopsis Stock Centre (NASC) along with ABRC (Arabidopsis Biological Resource Center) in the stores, maintains and distributes over 500,000 lines. Our stocks include a large proportion of insertion mutation lines approaching saturation of the transcriptome, ecotypes, mapping lines, activation tagged inserts, and promoter/enhancer traps.

New lines include Gabi Kat lines, Agrikola RNAi silencing seed and clones, and TAMARA activation tag lines. Confirmed homozygous T-DNA insertion lines from the SALK institute and from other groups are now being distributed. A number of smaller donations have also been received from a number of groups as a result of the stock centres' recent donations request.

All of these seeds can be ordered through our online catalogue at http://arabidopsis.info

87 High-density *Arabidopsis* Protein Arrays – a First Step Towards the Development of the *Arabidopsis* Interactome

Sorina Popescu, George Popescu, Shawn Bachan, Zimei Zhang, Michael Snyder, S. P. Dinesh-Kumar
Department of Molecular, Cellular and Developmental Biology, 266 Whitney Avenue, New Haven, CT 06520-8103

Much emphasis has been placed on the analysis of eukaryotic genomes at the level of nucleic acids. However, these studies alone are not sufficient to predict the structure, function, and activity of the proteins. Proteomics, the global analysis of proteins, is emerging as an important area of research in the post-genome era. As with the case of DNA chips, the development of a matrix with bound purified protein in addressable grids would greatly facilitate the analysis of large numbers of protein samples. We are generating a collection of high quality expression clones of *Arabidopsis* ORFs as tandem affinity purification (TAP) tag fusions. Using these clones, we optimized a transient plant protein expression system to produce and purify *Arabidopsis* proteins in a high-throughput manner. The first high-density *Arabidopsis* protein arrays containing 1152 unique recombinant protein preparations produced and purified from an homologous system, were used for the identification of binding partners for the Ca^{2+} sensor protein, calmodulin.

Here we present a comparative protein interaction analysis of seven *Arabidopsis* calmodulin isoforms and calmodulin-like proteins and their protein array targets. Our screen identified a complex interaction network of well-known and novel calmodulin-interacting partners. These results expand established calmodulin functions and also suggest a broader role for this protein in regulating cellular processes in plants. Our efforts establish protein arrays as a suitable platform for protein-protein interaction experiments that could potentially be applied to the whole proteome.

88 Development of a Small-scale, High-performance Microarray for Detailed Characterization of the Arabidopsis Response to Pathogen Attack

Masanao Sato[1], Raka Mitra[1], John Coller[2], Dong Wang[3], Natalie Weaver[3], Xinnian Dong[3], Jane Glazebrook[1], Fumiaki Katagiri[1]
[1]Dept. of Plant Biology, University of Minnesota, [2]Stanford Functional Genomics Facility, [3]Dept. of Biology, Duke University

In response to pathogen attack, Arabidopsis undergoes dynamic transcriptional reprogramming, controlled by a complex signaling network. To elucidate the structure of the signaling network, we need to specifically perturb this network and then collect detailed descriptions about it's state. Gene expression profiling can be used as a highly parallel phenotyping method to collect detailed descriptions of the network state. Current commercial or custom microarray platforms are unsuitable for this analysis either due to the high cost or low technical reproducibility. We report the development of a high-performance small-scale microarray platform, or "miniarray", for systems analysis.

To make large-scale systems analysis projects possible, we solved two major problems associated with current small-scale microarray experiments: poorly established statistical approaches and the lack of expandability due to the expression ratio-based measurements. Because statistical methods developed for large-scale microarrays are typically inappropriate for small-scale microarrays, we developed a new normalization method and probe-sharing subarray group pattern that allows the use of a statistical model to correct systematic biases. Conventional ratio-dependent measurements using two-color microarrays require the comparison of paired samples in a hybridization, restricting comparison between unpaired samples. To allow for comparison between unpaired hybridization samples, we implemented a calibration probe to measure the expression values for a particular sample instead of the expression ratio between two samples.

The resulting microarray showed excellent performance. The correlation coefficients between technical duplicates ranged from 0.979 to 0.996. A comparison of data obtained from the miniarray and the Affymetrix ATH1 GeneChip showed a correlation coefficient of 0.88. The accuracy of the miniarray was further supported by quantitative RT-PCR experiments and data from of spiked control RNAs. Using this miniarray, we initiated the analysis Arabidopsis mutants to elucidate the signaling network of RPS2-mediated resistance, highlighting transcriptional differences between mutants that are otherwise phenotypically indistinguishable.

89 Acquisition and Distribution of Stocks for Genomics and Phenomics by ABRC

Randy Scholl, Luz Rivero, Deborah Crist, Emma Knee, Natalie Case, Heather Joesting, Daniel Johnson, Kate Ludwig, James Mann, Cori Phillips, Garret Posey, Pamela Vivian, Zhen Zhang, Ling Zhou

Arabidopsis Biological Resource Center, Department of Plant Cellular and Molecular Biology and Plant Biotechnology Center, The Ohio State University

The Arabidopsis Biological Resource Center (ABRC) maintains many stocks relevant to genome exploration and functional genomics. Several flank-tagged insertion collections are distributed by ABRC, specifically: the T-DNA lines from a) SALK Institute, J. Ecker lab, b) Syngenta Biotechnology, including the ca. 9,000 lines, originally associated with MTAs, c) the Wisconsin Ds-Lox population, d) GABI-Kat lines, and e) RNAi lines from AGRIKOLA.

Developing initiatives related to insertion lines and their application to phenomics include: a) receipt of 1,900 confirmed, purified insertion lines from J. Ecker; b) efforts to make these lines available for large phenotypic studies; and c) regrowth of the entire ABRC collection of natural accessions to reestablish single seed sources which will be genetically fingerprinted by J. Borevitz and collaborators.

Full length open reading frame (ORF) cDNA clones are still being received. The total number of full length clones is approximately 15,000 including donations from SSP and Salk (J. Ecker), TIGR (C. Town), Peking/Yale and J. Callis. In addition, we are receiving a large collection of ORF clones cloned into Gateway ™ Expression constructs – 1,100 of these (from S. P. Dinesh Kumar) are currently in house. Versatile Destination vectors (for Gateway ™ and other systems) for various expression applications in plants, bacteria and yeast are available. We have formatted 11,000 of the new cDNA clones into plates. We plan to distribute other similar large collections in this way.

ABRC is supported by the National Science Foundation.

90 BRET: A Method to Detect in vivo Protein Interactions in Plant Cells

Chitra Subramanian, Jongchan Woo, Albrecht von Arnim

University of Tennessee, Knoxville

Sensing extracellular signals and transmitting them into specific responses involves protein-protein interactions within a cell. In order to detect protein-protein interactions in vivo we have used Bioluminescence Resonance Energy transfer (BRET). BRET involves radiation-less energy transfer between Renilla luciferase (RLUC) and YFP if the candidate fusion protein partners interact with each other. We have generated a suite of BRET expression vectors for both restriction cloning and gateway cloning. A codon optimized version of RLUC enhanced the stable expression of fusion proteins in transgenic Arabidopsis. BRET has been demonstrated between a B-box protein, STH, and an E3 ubiquitin ligase, COP1 and also between two b zip transcription factors HY5 and HYH in stable transgenic Arabidopsis seedlings. RLUC can be targeted to a variety of subcellular organelles like chloroplasts, mitochondria, golgi and peroxisomes. Finally, we have also demonstrated the use of Renilla luciferase as a reporter of protein stability in a cycloheximide chase assay. Results will be presented for a survey of pair wise protein-interactions thought to play a role in the Arabidopsis light signaling network.

91 Approaches to Study Homologous Recombination in Arabidopsis

Erica Unger-Wallace, Xianyan Kuang, David Wright, Daniel Voytas, Thomas Peterson

Department of Genetics, Development & Cell Biology, Iowa State University, Ames, IA, USA

There are many tools available to the Arabidopsis research community for investigations of gene function. However, a critical capability currently lacking is a means to edit specific gene sequences using homologous recombination, or gene targeting. Our strategy is to utilize enzymes, such as Ac transposase, and gene-specific zinc finger nucleases to generate a double strand break (DSB) at target chromosomal loci. Such locus specific breaks are known to stimulate recombination in many systems, including plants. In addition we are testing whether expression of the yeast Rad54 protein, together with a DSB, induces a higher recombination frequency than either of these factors alone with the goal of developing an efficient approach to gene targeting in Arabidopsis. Model transgenic target loci have been generated that contain a defective GUS-NPTII gene interrupted by either a non-autonomous Ds transposable element or the recognition sequence for a zinc finger nuclease. Donor DNA, designed to repair the gene and restore function and either Ac transposase or the zinc finger nuclease, are introduced by Agrobacterium-mediated transformation. Putative recombinant plants are selected using a kanamycin resistant phenotype conferred by correction of the defective reporter gene. GUS expression and molecular analysis of these plants serves to characterize the types of recombination events recovered. Similarly, native Arabidopsis loci with Ds insertions have been selected and will be examined to determine the frequency of recombination across several locations in the Arabidopsis genome.

92 Characterization of Arabidopsis mRNA Polyadenylation Machinery: Genetic and Biochemical Analysis of yPcf11p Homologous

Denghui Xing, Min Mo, Q. Quinn Li

Department of Botany, Miami University, Oxford, Ohio 45056

The 3'-end cleavage and polyadenylation of eukaryotic pre-mRNA involves several protein complexes containing more than a dozen of subunits. Yeast (S. cerevisiae) Pcf11p is one of these subunits and is essential for appropriate 3'-end processing. yPcf11p interacts with Rna14p, Rna15p and ClpIII subunits, formimg the CFIA complex of polyadenylation machinery. In addition, through interacting with CTD domain of RNA Pol II largest subunit, yPcf11p is also a factor for transcription termination. The biochemical and biological function of yPcf11p is thought to be largely conserved between yeast and mammals. However, little is known about plant counterpart of yPcf11p. We have identified a small gene family of Arabidopsis Pcf11p homologous and other components of CFIA. To elucidate the significance of these Arabidopsis proteins in polyadenylation and transcription termination, we have tested their interaction with other polyadenylation factors using yeast two-hybrid and in vitro pull-down assays. Analysis of T-DNA knockouts of Arabidopsis genes encoding homologous of yPcf11p revealed potential interesting phenotypes. The results will be presented.

93 Reverse Genetic Analysis of the F-box Protein Family in Arabidopsis

Xiaohua Zheng, Richard Vierstra

Department of Genetics, University of Wisconsin-Madison

The growth and development of Arabidopsis are delicately tuned by the synthesis of nascent protein as well as degradation of undesirable protein. The breakdown of many regulatory proteins is accomplished by the ubiquitin (Ub) 26S proteasome system. Through an ATP-dependent enzymatic cascade involving E1s, E2s and E3s, Ub becomes covalently attached to various short-lived proteins, then these ubiquitinated species are recognized and degraded by the 26S proteasome. A large family of E3s (or Ub-protein ligases) is responsible for choosing appropriate substrates for turnover. One type of E3s, the SCF complex, is composed of cullin, Rbx1, Skp1(Ask) and F-box protein subunits, with the F-box protein serving as the substrate recognition factor. We previously identified almost 700 loci in Arabidopsis that encode F-box proteins, making this family one of the largest in plants. While a number have been shown to control important developmental and physiological processes in plants, the functions of most F-box proteins are unknown. To help define these function(s), we have generated a large collection of T-DNA insertion mutants in F-box genes that previously have not t been studied in Arabidopsis. In total, we screened 249 Salk lines and obtained useful mutants for 104 loci. We failed to obtain homozygous mutants for only two lines, indicating that a majority of the Arabidopsis F-box protein genes are not essential. Phenotypic analysis of the remaining 104 revealed little or no defects under standard growth conditions. However, mild phenotypes were detected for some mutants when exposed to various conditional treatments. Preliminary analyses of several have revealed roles for F-Box proteins in sugar metabolism and various hormone responses. Further analysis of this population is ongoing with the aim of comprehensively understanding the functions of key F-box proteins in plant development.

with meals

94 Purification and Biochemical Characterization of Two beta-Thioglucoside Hydrolysases (TGG1 and TGG2) in Arabidopsis thaliana

Changhe Zhou[4], David Bevan[2], Michael Reichelt[3], Asim Esen[1]

[1]Department of Biology, Virginia Tech, [2]Department of Biochemistry, Virginia Tech, Blacksburg, VA24061, [3]Department of Biochemistry, Max-Planck Institute, Jena, Germany, [4]Department of Biology, Virginia Tech, Blacksburg, VA24061

The myrosinase-glucosinolate system is a major pre-formed chemical defense system involved in a range of biological interactions between insects, pathogens and host plants of the Brassicaceae. Myrosinases (beta-thioglucoside hydrolysase) catalyze the hydrolysis of glucosinolates, a group of nitrogen- and sulphur-containing secondary metabolites. Native myrosinase from white mustard seed forms a dimer stabilized by a Zn2+ ion, is heavily glycosylated and folds into a (b/a)8-barrel structure. There are several functional sites in the native myrosinase including binding sites for ascorbate, glucosinolate, water, Zn2+ and myrosinase-binding proteins. Four myrosinase genes, namely TGG1 (At5g26000), TGG2 (At5g25980), TGG4 (At1g47600) and TGG5 (At1g51470), as well as two pseudogenes, have been identified in Arabidopsis. There is little or no information available about purification and biochemical characterization native myrosinases from Arabidopsis. To better understand the function and biochemistry of myrosinases, TGG1 and TGG2 homozygous T-DNA insertion lines have been selected and confirmed with Western analysis using peptide-specific antibodies, which showed that TGG1 and TGG2 expression were completely blocked in T-DNA lines. In comparison with wildtype, no abnormal phenotypes were observed in TGG1 and TGG2 T-DNA lines. Western blot analysis showed that TGG1 and TGG2 were strongly expressed in seedling, rosette leaf, cauline leaf, stem, flower and silique while specific activity in different organs showed that TGG1 has higher activity than TGG2 in those organs. Both TGG1 and TGG2 expression could be induced by methyl jasmonic acid and Pseudomonas syringae. The homogeneous TGG1 and TGG2 were isolated from Arabidopsis plants and characterized, including kinetics and substrate specificity. More than 90% of TGG1 in Arabidopsis plants is soluble, whereas least 60% of TGG2 is insoluble. High pH and salts enhanced the solubility of TGG2. Native TGG1 is a dimer with a molecular mass of 150kD and a monomer with a molecular mass of 73kD. TGG1 and TGG2 exhibited a wide range of substrate specificity, they also had beta-glucosidase activity and hydrolyzed p-nitrophenyl beta-D-glucopyranoside(pNPG), o-nitrophenyl beta-D-glucopyranoside(oNPG) and 4-methylumbelliferyl-beta-D-glucopyranoside(4-MUG). TGG1 could be activated in the presence of ascorbate while TGG2 was inhibited by ascorbate.

This project has been supported by NSF Arabidopsis 2010

95 Predicting the Redundome: A Genome-Wide View of Overlapping Gene Function

Dennis Shasha, Sunayan Bandyopadhyay[1], Cindy Yang[2], Kenneth Birnbaum[2]

[1]Courant Institute, New York University, [2]Biology Department, New York University

High throughput analysis of gene function in plants will be accelerated by three sets of resources: 1) an intricate knowledge of when and where genes are active, 2) predictive power to define genetic redundancy, and 3) facile techniques to knockout multiple genes and observe their phenotype. The availability of thousands of microarray experiments in Arabidopsis has provided progress on the first resource. We use, in part, the comprehensive expression to report progress on the second resource, predicting genetic redundancy in a model organism replete with high levels of gene duplication. Using a training set of documented cases of redundant and non-redundant genes, we find that commonly used attributes to determine genetic redundancy, such as sequence comparison or gene expression, are poor predictors of redundancy alone. To address this issue, we combined a wide set of attributes for potential gene pairs in the genome, including sequence comparison, thousands of microarray experiments, the sharing of predicted protein domains, and others. Considering multiple attributes of gene pairs together, we have used the training set and the list of attributes to guide two machine learning techniques -- decision trees and support vector machines -- to establish non-trivial rules to predict functional overlap in the Arabidopsis genome. Withholding analysis shows that these rules are 75 to 80% accurate. This provides a critical resource in devising a systematic methodology to plan multiple mutant experiments to uncover functional roles of Arabidopsis genes.

96 Web services for Arabidopsis data integration

Dirk Haase[1], Hank Wu[2], Heiko Schoof[1], Chris Town[2]

[1]Max-Planck-Institute for Plant Breeding Research, Cologne, Germany, [2]The Institute for Genomic Research, Rockville MD, USA

In an international collaborative project, web services for Arabidopsis data integration are being generated using the BioMoby platform. These will allow novel queries and analyses and are especially suited to generate custom workflows. A project web site provides an entry point to a collection of workflows and tools to query and use the web services network. It also provides developer resources aiming to make it easy for new data providers to join the project and develop and integrate their resources. Online demonstrations will be available at the poster. In a scheduled workshop, progress in the project and the tools and services available will be presented. We are very much interested in feedback and input on web services, tools or workflows that would be important for the community, and will be collecting these at the poster and at the workshop.
http://bioinfo.mpiz-koeln.mpg.de/araws

97 Applications of Plant Ontologies for Describing and Comparing Phenotypes and Gene Expression in Plant Databases

Katica Ilic[1], Elizabeth Kellogg[2], Peter Stevens[3], Felipe Zapata[3], Pankaj Jaiswal[4], Anuradha Pujar[4], Shulamit Avraham[5], Leszek Vincent[6], Leonore Reiser[1], Marty Sachs[7], Susan McCouch[4], Mary Schaeffer[6], Doreen Ware[5], Lincoln Stein[5], Seung Rhee[1]

[1]TAIR, Carnegie Institution, Department of Plant Biology, Stanford, CA, [2]Department of Biology, University of Missouri-St. Louis, MO, [3]Botanical Garden, University of Missouri - St. Louis, MO, [4]Gramene, Deptartment of Plant Breeding, Cornell University, Ithaca, NY, [5]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, [6]MaizeGDB, University of Missouri - Columbia, MO, [7]Maize Genetics Cooperation - Stock Center & Department of Crop Sciences, University of Illinois, Urbana, IL

The Plant Ontology Consortium (POC) was established as a collaboration among several plant databases and experts in plant systematics, botany and genomics. The primary goal of POC was to create a semantic framework for meaningful cross-species queries across plant databases and to facilitate and accommodate functional annotation efforts in plant databases and by the plant research community at large. Thus, the POC has created controlled vocabularies (ontologies) which describe morphology, anatomy, growth and developmental stages of a flowering plant. Plant Ontology (PO) has two aspects: 1) Plant Structure Ontology, with terms describing organs, organ systems, tissues, and cell types; and 2) Plant Growth and Developmental Stage Ontology, with terms describing spatial-temporal growth stages of a whole plant and developmental stages of organs and organ systems. The initial releases of Plant Ontology included integration of existing ontologies for Arabidopsis, maize and rice, thus spanning the eudicot-monocot divide. Recently, we have expanded PO to encompass Fabaceae, Solanaceae and other cereal crops. Consistent use of these ontologies potentially reduces the problem of heterogeneity of terminology used to describe comparable object types in plant databases - an obvious obstacle for conducting queries for multiple species across plant genomic databases. As a part of ongoing functional annotation efforts, participating databases, such as TAIR, NASC, Gramene, MaizeGDB and SOL Genomics Network, have been using PO to describe expression patterns of genes and phenotypes of mutants and natural variants. Close to 10,000 gene annotations and phenotype descriptions from several species-specific databases can now be queried and retrieved using the Plant Ontology browser. A summary of the organizing principles and rules followed in developing PO will be presented, as well as the annotation examples from member databases using PO terms. The PO and gene and phenotype associations contributed from member databases can be obtained at www.plantontology.org.

98 Report on plant resource project in RIKEN BRC

Masatomo Kobayashi, Hiroshi Abe, Satoshi Iuchi, Toshihiro Kobayashi

RIKEN BRC

RIKEN BRC preserves and distribute plant resources such as seeds for Arabidopsis transposon-tagged lines (KO-line) and Arabidopsis full-length cDNA (RAFL) clones that have been developed in Japan. During last year, RIKEN BRC almost finished the preparation of these important materials for distribution. We are now establishing homozygous seed stocks of the transposon-tagged lines that will be partially available within the end of this year.

Now approximately 366,000 materials are preserved in RIKEN BRC, and more than 18,000 materials have been shipped to world research community. Our stock include Arabidopsis transposon-tagged and activation (T-DNA)-tagged lines, seed lines for natural accessions, RAFL clones and full-length clones of some model plant species such as Physcomitrella patens (model moss) and Populus nigra (model tree). The detail information is available from our web site: http://www.brc.riken.jp/lab/epd/Eng/.

99 ReIN: an interactive tool to create and visualize regulatory networks in Arabidopsis thaliana

Saranyan Palaniswamy, Sarat Subramaniam, Rebecca Lamb, Ramana Davuluri, Erich Grotewold
The Ohio State University

ReIN is a web-based software tool for creating, visualizing and identifying regulatory networks in *Arabidopsis thaliana*. Regulatory motifs represent the simplest units of the overall regulatory network of an organism. A simple graphical interface tool like ReIN will facilitate biologists a fast and easy way to visualize networks of interactions in *Arabidopsis* with the potential to be extended to other organisms. The identification of the direct targets for TFs is a first step in establishing the regulatory motifs in which particular TFs are involved. *Arabidopsis* is a small dicotyledoneous plant for which the entire genome is available. The *Arabidopsis Gene Regulatory Information Server (AGRIS,* http://arabidopsis.med.ohio-state.edu) provides a comprehensive collection of all known *Arabidopsis* TFs, the promoters, and known and predicted interactions between the TFs and the corresponding promoters. For a complete understanding of the intricate behavior of these gene regulatory networks, the development of web-based computational tools becomes essential (Blais and Dynlacht, 2005). The visualization of a network of regulatory interactions between different TFs and genes consists of translating interaction data into 2D images where different participants in the regulatory chain are represented by different geometric entities. The visualization process may therefore be divided into three steps or models: extracting information from a database consisting of TF or gene interactions; mapping these data into various geometrical shapes and arrows, representing the interactions; and finally, laying out and drawing these shapes while providing the user ability to control and interact with the drawn network in various ways. ReIN is a web-based application with a multi-tier architecture based on J2EE technology. The application is available to the user as a Java applet embedded in JSP which provides the user the ability to search for TFs or genes in the *Arabidopsis thaliana Regulatory Network Database* (*AtRegNet)*. ReIN uses java servlets to retrieve information and allows the user to upload input (network) data as a file and download the network visualization as an image. This process allows a large number of users to simultaneously interact with the database without a noticeable impact on performance http://arabidopsis.med.ohio-state.edu/REIN.

100 ARGOS, a simple and one-step SSR finder for supporting molecular marker development in plant researches

Kyu-Won Kim[1], Jae-Woong Yu[2], Hun-Ki Chung[3], Kyung-Ho Ma[3], Tae-San Kim[3], V. Ramanatha Rao[4], Yong-Jin Park[4]
[1]Qube Soft Inc., [2]Seoul National University, Seoul, R. Korea, [3]National Institute of Agricultural Biotechnology, Suwon, R. Korea, [4]International Plant Genetic Resources Institute (IPGRI-APO), Serdang, Malaysia

SSR marker system technology is useful for comparing genetic characteristic of subspecies and finding markers related to phenotypes of economic importance and mapping genes through construction of linkage map. Since computer power can help searching simple sequence repeats (SSRs) quickly in the sequence data read from the mass DNA fragments, there are many algorithms developed for helping that. But it is very complicated to modify output results obtained from one program to input format of another program. This situation would be more serious when handling many clone sequences, at a time and this is very hard work to manage mass data read from DNA fragment sequences and their data output after analysis. Therefore, I developed one step computer program for rapid identification of simple sequence repeats and primer design which is coded with C# and Microsoft Visual Studio.NET. Distant parser sorts the SSR motifs from local N repeat parser and merges neighboring SSRs to same motif for primer design. We compared this program with 4 other SSR finding programs (Mreps, SSRIT, Sputnik, Troll) with 320 clone sequences of 8 different plant species. We found increased accuracy and outstanding outputs of this program, compared with other currently used programs.

This research was conducted through IPGRI and RDA (Rural Development Administration, R. Korea) collaboration, supported by BioGreen 21 Program.

101 An "electronic Fluorescent Protein" Browser for Exploring Arabidopsis Microarray Data

Ben Vinegar[1], Debbie Winter[2], Nicholas Provart[3]

[1]Dept. of Computer Science, University of Toronto, Toronto, ON. CANADA, [2]Bioinformatics and Computational Biology Program, Univesity of Toronto, Toronto, ON. CANADA, [3]Dept. of Cell and Systems Biology, University of Toronto, Toronto ON. CANADA

We have developed a web-based tool for exploring Arabidopsis microarray data and have dubbed it the Arabidopsis electronic Fluorescent Protein, or eFP, Browser. The eFP Browser permits intuitive visualization of gene expression data across approximately 22,000 genes from Arabidopsis thaliana, as represented on the ATH1 GeneChip from Affymetrix. Currently, the expression data for the eFP Browser come from Schmid et. al.'s Gene Expression Map of Arabidopsis Development (2005, Nature Genetics 37:501-6), and we mirror these data in the Botany Array Resource (Toufighi et al., 2005, Plant J. 43:153-63) for quicker access. The user is presented with an idealized image of Arabidopsis whereby plant tissues are coloured according to the expression level of the user's gene of interest, in three useful interpretive modes – absolute, median and compare. The tool is intended as a quick and easy means of identifying tissues of interest, and is particularly useful when exploring gene families to facilitate hypothesis generation. It is our goal to make this tool into a community resource whereby researchers from around the world can upload both data sets and diagrammatic representations of the experiment in question. Users of the resource will then be able to explore microarray experiments by examining compact representations of the experiments overlaid with gene expression data. The eFP browser is available at http://bbc.botany.utoronto.ca/efp/.

102 A massively parallel genome survey of soybean

Kranthi Varala, Kankshita Swaminathan, Raymond Rouf, Matthew Hudson

University of Illinois

A microbead-based sequencing technology was used to generate 717,383 genomic survey sequences, averaging 112 base pairs in size, from soybean (Glycine max) cv. Williams. These sequences represent an estimated 7% of the genome of soybean, dispersed at average 2Kb intervals in single copy regions of the soybean genome. 10,464 of the reads could be identified as derived from likely genomic protein coding regions. 41% of the conceptual translations of these regions are not known soybean protein sequences, giving over 4,000 novel proteins or protein fragments. The survey lacks cloning bias, and it is therefore possible to use these sequences to reconstruct a representative dataset of soybean repetitive sequences *in silico* on a whole-genome scale. Over 30,000 multi-copy sequences, of which 4213 are present in 100 or more copies per genome, were detected using clustering and assembly of the short sequences. These include transposons, satellites, centromeres and telomeres. We have collated, curated and annotated these repeats and developed an on-line database where these sequences can be accessed and searched. Using this database, we estimate that 41% of the soybean genome is present in more than 14 copies per haploid set, in agreement with Cot measurements.

103 Proteomic services at the European Bioinformatics Institute

D Thorneycroft, J Khadake, S Orchard, L Montecchi-Palazzi, S Kerrin, C Leroy, B Aranda, M Kleen, P Jones, R Cote, L Martens, A Quinn, W Derache, C Taylor, A Fleischmann, M Darsow, K Degtyarenko, W Fleischmann, S Boyce, K Axelsen, J Risse, M Ennis, N Vinod, P de Matos, S Klie, H Hemjakob, R Apweiler

European Bioinformatics Institute

Proteome analysis is becoming a powerful tool in the functional characterization of plants and has been applied to processes such as photomorphogenesis, flower development, protein degradation systems, signal transduction and plant-microbe protein interactions. These studies have expanded and refined understanding of the plant proteome and interactome in both model and crop species.

The European Bioinformatics Institute (EBI) is the European node for globally coordinated efforts to collect and disseminate biological data. The Proteomics Services Team (EBI) provides databases and tools for the deposition, distribution and analysis of proteomics and proteomics-related data. PRIDE, the 'PRoteomics IDEntifications database' (http://www.ebi.ac.uk/pride) is a database of protein and peptide identifications that have been described in the scientific literature. These identifications may be annotated with supporting mass spectra. PRIDE is a web application, so submission, searching and data retrieval can all be performed using an internet browser. The Intact database (http://www.ebi.ac.uk/intact/index.jsp) provides a freely available, open source database system and tools for the storage and analysis of protein interaction data. The IntAct toolkit provides textural and graphical representation of the data and allows the user to explore protein interaction networks using a web-based interface. The database contains an increasing amount of protein interaction data derived from model and crop plants.

The Proteomics Services team coordinates HUPO's Proteomics Standards Initiative (PSI). , which is developing data standards for protein-protein interactions, mass spectrometry and general proteomics. The proteomics team also provides biochemical reference databases such as IntEnz, the Integrated relational Enzyme database which is the most up-to-date version of the Enzyme Nomenclature and ChEBI, the Chemical Entities of Biological Interest database, a freely available dictionary of molecular entities focused on 'small' chemical compounds. The Proteomics Services Team seeks to constantly evolve to meet the needs of its users and play its part in the development and adoption of proteomic standards by the scientific community. For more information visit http://www.ebi.ac.uk.

PRIDE: a public repository of protein and peptide identifications for the proteomics community. P Jones et al. Nucleic Acids Research Vol 1 Issue 34 (Database issue) D659-D663. IntAct - an open source molecular interaction database. H. Hermjakob et al. Nucl. Acids. Res. 2004 32: D452-D455. IntEnz, the integrated relational enzyme database. Fleischmann A et al. Nucleic Acids Res. 2004 Jan 1;32(Database issue)D434-7. Autumn 2005 Workshop of the Human Proteome Organisation Proteomics Standards Initiative (HUPO-PSI) Geneva, September, 4-6, 2005. S. Orchard et al. Proteomics. 2006 Feb;6(3):738-41. The European Bioinformatics Institute's data resources: towards systems biology. Brooksbank C et al. Nucleic Acids Res. 2005 Jan 1;33(Database issue):D46-53.

104 New Gene Discovery in Unannotated Regions of the Arabidopsis Genome

Yongli Xiao, William Moskal, Hank Wu, Beverly Underwood, Wei Wang, Erin Monaghan, Christopher Town

The Institute for Genomic Research, Rockville MD, USA.

The whole genome sequence of Arabidopsis with its annotation was completed at the end of 2000. Since then, four versions of genome re-annotation were released sequentially based on new experimental evidence and improved computational analysis. Evidence based mainly on sequence conservation indicated the existence of yet more genes in un-annotated regions of the genome. TwinScan and EuGene are two relatively new gene prediction programs that incorporate comparative genomic information. Compared with the TIGRv5 annotation, EuGene predicts 1,559 and TwinScan predicts 1,440 genes in intergenic regions of the Arabidopsis genome with 365 predictions in common. In order to verify the novel genes predicted by EuGene and TwinScan, a high throughput method of rapid amplification of cDNA ends (RACE) using cDNA from 11 diverse RNA populations was applied to 918 predictions in intergenic regions. We recovered transcripts from 429 predictions that yielded 323 novel full-length cDNAs. In addition, a comparative study of Arabidopsis and Brassica yielded a number of Conserved Arabidopsis Genome Sequences (CAGS), 28% of which aligned to un-annotated regions suggesting the presence of un-annotated novel genes. We similarly targeted 192 intergenic CAGS by the RACE pipeline and found an additional 25 un-annotated genes. Thus, a total 454 of novel genes in the intergenic regions of Arabidopsis has been recovered. We have implemented a streamlined Gateway® cloning protocol that permits simultaneous generation of ORF clones with and without stop codons with little additional cost. To date, we have generated ORF clones in Gateway® entry vectors for over 2,000 Arabidopsis genes, including the novel intergenic structures described above, that are expressed at very low levels and thus absent from current EST/cDNA collections.

Research supported by the NSF 2010 Project.

105 "MobyMan" a Light Weight Biomobyed MapMan Server Application

Bjorn Usadel[1], Axel Nagel[2], Dirk Steinhauser[1], Henning Redestig[1], Oliver Blaesing[1, 3], Mark Stitt[1]

[1]Max-Planck Institute of Molecular Plant Physiology, System Regulation, Am Muhlenberg 1, D-14476 Golm, [2]RZPD Deutsches Ressourcenzentrum fur Genomforschung GmbH, Heubnerweg 6, D-14059 Berlin, [3]Present Address: Metanomics, Tegeler Weg 33, 10589 Berlin

MapMan is a stand alone java application for the visualization of large datasets that arise from various profiling experiments (see Abstract #9317). These datasets are visualized on pre-drawn maps which represent biological pathways or overview pictures.

Even though a webversion of MapMan exists (http://www.gabi.rzpd.de/projects/MapMan/) this is based on the JAVA technology, and thus requires a more potent platform when installing it on a web server.

However, since most small web servers are scripting enabled by default, we present here a completely new implementation of MapMan in Perl which can be easily plugged into existing server sided analysis platforms for data visualization. This implementation allows uploading of data which is then immediately displayed by the server. To further facilitate interoperability of this new tool it has been equipped with a BioMoby interface for communication with other biological databases.

The Biomoby interface allows both to access other third-party databases and to move data from these databases for display into this “biomobyed” MapMan version.

As an exemplary case a novel microarray database extension to CSB.DB / MapMan and its interplay with the new MapMan version will be discussed in detail.

Special thanks are due to Dr. Heiko Schoof for promoting and advocating the Biomoby interface.

106 Combinatorial Analysis of Cis-elements and Gene Expression to Identify Co-chaperones for *A. thaliana* HSP90 Genes

Cecilia Vasquez-Robinet[1], Amrita Pati[2], T. Murali[2], Lenwood Heath[2], Hans Bohnert[3], Ruth Grene[1]

[1]Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA, [2]Computer Sciences, Virginia Tech, Blacksburg, VA, [3]Plant Biology, UIUC, Urbana-Champaign, IL

Exposure to mild drought stress can "pre-condition" the plant photosynthetic machinery, "acclimating" it to more successfully defend itself against subsequent drought stresses. We have shown that specific expression patterns of genes encoding several heat shock proteins (HSPs) are correlated with photosynthetic acclimation under mild drought stress in loblolly pine (1,2). The experiments showed that a homolog of Arabidopsis HSP90-7 was up-regulated after rehydration coincident with acclimation (2). HSP90-7 is a member of the HSP90 gene family that in mammals forms a complex with co-chaperones involved in activation, processing or trafficking of signaling proteins. We found that the HSP90-7 homolog was co-expressed with candidate co-chaperones such as HSP70-3, and a HSP40 homolog. Homologs of these proposed co-chaperone genes are present in *A. thaliana* as large multigene families. The evolution of these large gene families in plants can be rationalized by the necessity of adaptation to a range of stresses, since HSPs are induced not only under heat but also under other abiotic stresses, such as drought, as we observed in loblolly pine. Therefore, adaptation might involve the formation of specific chaperone complexes for different stresses but a demonstration of their existence in plant systems is still lacking.

Should plants produce specific chaperone complexes to signal and defend against abiotic stress, we would expect similar arrangements of cis-elements in the upstream regions of plant HSP90s and their particular co-chaperones to guarantee stress-dependent co-expression. Analysis of the promoter region of *A. thaliana* HSP90s and their putative co-chaperones with XcisClique (3) resulted in the identification of candidate co-chaperones for each HSP90. Some of the candidate co-chaperones found in the Arabidopsis genome for HSP90-1 and HSP90-7 are homologs of the pine genes that were co-expressed in our drought experiment. We are performing yeast two hybrid experiments with candidate co-chaperones to confirm the predictions found by XcisClique.

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107 Ubiquitin Lys 63 Chain Forming Ligases RGL51 and RGL52 Mediate Apical Dominance, Hormone Balance and Cell Fate Decisions in *Arabidopsis*

Xiao-Jun Yin[1], Sara Volk[2], Karin Ljung[3], Karel Dolezal[3], Shigeru Hanano[1], Seth Davis[1], Elmon Schmelzer[1], Goran Sandberg[3], Cecile Pickart[2], Andreas Bachmair[1]

[1]Max Planck Institute for Plant Breeding Research, D-50829 Cologne, Germany, [2]Johns Hopkins University, Baltimore, MD, USA 21205, [3]Umea Plant Science Centre, S-90183 Umea, Sweden

Polyubiquitin chains assembled through Lys 48 of ubiquitin are recognition signals for degradation of the modified substrate protein. In contrast, chains assembled through Lys 63 of ubiquitin have no function in proteolysis. A known role for ubiquitin Lys 63 chains in animals and fungi is in DNA repair. The function of these chains in plants is unclear at present.

In order to investigate ubiquitin Lys 63 chain formation in Arabidopsis, we carried out a yeast two-hybrid experiment, using the plant homologs of two conserved cofactors of ubiquitin Lys 63 chain formation, MMS2 and UBC13, as a bait. Two closely related ubiquitin protein ligases were identified, the RGL51 and RGL52. They reside at the plasma membrane and on the endo-membrane system. In vitro, RGL52 can attach ubiquitin Lys 63 chains onto itself. Double mutants *rgl51 rgl52* have altered levels of the mobile plant growth regulators, auxin and cytokinin. Response to exogenously added auxin is decreased compared to wild type plants. Mutants are bushy, and differ from wild type in a variety of additional traits such as phyllotaxy, circadian rhythm and cell size. RGL51 / 52 may be involved in signal transduction, for instance by influencing auxin and / or cytokinin transport and distribution.

108 The V-ATPase and its role in cell elongation

Angela Bruex, Matthias Grauer, Karin Schumacher

ZMBP Plant Physiology, University of Tuebingen, Germany

The vacuolar H+ ATPase (V-ATPase), a highly conserved eukaryotic proton pump present in the endomembrane compartments, plays a crucial role in establishing proton gradients, which are needed for secondary active transport and turgor regulation. The V-ATPase also functions in regulating the pH homeostasis of cellular compartments, which is important for protein targeting, enzyme activity, and vesicle trafficking. Inhibition of the V-ATPase with Concanamycin, a V-ATPase specific inhibitor leads to reduced hypocotyl growth, an effect also observed in the det3 (de-etiolated3) mutant. The det3 phenotype is caused by a mutation in the V-ATPase subunit C (VHA-C) leading to a reduced V-ATPase activity of approximately 50%. These pharmacological and genetical evidences indicate that the V-ATPase is important for cell elongation. To clarify the role of the V-ATPase in cell elongation, we further investigated the det3 mutant, an excellent tool due to its conditional phenotype: The det3 phenotype is inducible by nitrate and lower temperatures causing a short hypocotyl, whereas det3 seedlings grown under permissive conditions exhibit normal hypocotyl length. Further experiments like growth studies on different inhibitors, cellulose measurements and the investigation of the transcriptome revealed a similarity between det3 and cell wall synthesis mutants and a misfunction in vesicle trafficking.

109 MAP3Kepsilon1 and MAP3Kepsilon2 are Required for Normal Cell Expansion in Arabidopsis

Suraphon Chaiwongsar[1], Marisa Otegui[2], Patrick Krysan[1]

[1]Horticulture Department and Genome Center of Wisconsin, University of Wisconsin - Madison, Madison, WI 53706, USA., [2]Department of Botany, University of Wisconsin - Madison, Madison, WI 53706, USA.

We have used reverse-genetic analysis to investigate the function of MAP3Kepsilon1 and MAP3Kepsilon2, a pair of closely-related Arabidopsis thaliana genes that encode protein kinases. Plants homozygous for either map3ke1 or map3ke2 display no apparent mutant phenotype, whereas the double-mutant combination causes pollen lethality. We have previously demonstrated that double-mutant pollen grains develop plasma membrane irregularities following pollen mitosis I, and that MAP3Ke1 localizes to the plasma membrane. To study the function of MAP3Ke1 and MAP3Ke2 in somatic tissues, we employed an ethanol-inducible system to conditionally rescue double-mutant pollen grains. Using this approach we were able to generate map3ke1;map3ke2 homozygous double-mutant plants that also carried an ethanol-inducible MAP3Ke1 transgene. When grown under non-inductive conditions, these double-mutant plants had significantly smaller rosettes, shorter primary roots, and fewer lateral roots than wild-type. Microscopic analysis suggests that cell expansion is reduced in the double-mutant plants. We also investigated the expression pattern of MAP3Ke1 through the use of a YFP:MAP3Ke1 translational fusion construct and observed that the protein is highly expressed in meristem tissues and lateral roots. Taken together, our results suggest that MAP3Ke1 may be involved in the regulation of cell expansion in Arabidopsis.

110 Investigating the role of SYP71 and related SNAREs in polarized secretion

Laura Conner[1], Sivani Paskaradevan[1], Valya Kovaleva[2], Anton Sanderfoot[1]

[1]University of Minnesota, [2]University of California Riverside

Secretion is the process by which organisms move proteins and other products made within the cell to the outside of the cell. There are two different kinds of secretion; constitutive and polarized. Constitutive secretion is the default pathway for proteins and occurs if the protein has no other signal, and happens equally to all sides of the cell. Polarized secretion is different in that the proteins being secreted are targeted to a specific side of the cell instead of all sides of the cell. In land plants a modified form of polarized secretion is performed when the cell plate is created. Despite the importance of polarized secretion in many different processes (signal transmission, pathogen defense, cell growth, cytokinesis), little is known about how this type of secretion is accomplished.

SNARE proteins are involved in vesicle trafficking in a very specific process of the fusion between a vesicle with the target organelle. SNAREs involve a v-SNARE on the donor organelle fusing to 3 t-SNAREs on the target organelle. This can be used to understand the formation of the cell plate as well as other organelles involved in the process and is useful in seeing which SNAREs interact. The SNARE system is fairly well understood and so can be valuable in the study of these processes.

The SYP7 family of SNAREs consists of three members and appears to hold essential roles. The three *SYP7*'s are found in different parts of the plant and expressed at different levels. By whole mount immunofluorescence, immunoprecipitation, and western blot, SYP71 has been shown to be localized to the cell plate, as well as a particular side of the plasma membrane in interphase root cells. Interestingly, this is the first plant SNARE to have this type of polarized localization at the PM of interphase cells. Pharmacological and EM results suggest that SYP71 is also found on endosomes. *SYP71* is found in moderate levels in most tissues of the plant, *SYP72* very low in most tissues but very high in pollen, and *SYP73* is found low in most tissues with a moderate level in pollen. Whereas *SYP73* mutants are phenotypically normal, mutants in *SYP71* have been found to be embryonic lethal, and *SYP72* mutants to be male gametophytic lethal; each consistent with an essential role in the cells in which they are expressed.

Due to the unique nature of the SYP7 family and the discovery of the first polarized plant SNARE SYP71, these proteins will provide a system to study polarized secretion and its role in growth, cell division, and development.

111 Characterization of the Anti-microtubule Drug Supersensitive Arabidopsis Mutant 28-2b

Charitha Galva[1], Alex Paradez[2], John Sedbrook[1]

[1]Department of Biological Sciences, Illinois State University, Normal, Illinois 61790., [2]Carnegie Institution, Department of Plant Biology, Stanford, California 94305.

Microtubules assemble into highly organized arrays that are essential for nuclear migration, cytokinesis, and cell expansion. In plants, microtubules form a cortical array that lines the periphery of each cell and plays a role in regulating and directing cell wall deposition and cell expansion. Little is known about the mechanisms underlying the organization of this array, or even how it carries out its functions. To learn more, we are taking a molecular genetic approach by characterizing an Arabidopsis mutant line 28-2b whose cortical microtubule arrays are hypersensitive to disruption by the anti-microtubule drug oryzalin. On agar growth medium containing 150 nanomolar oryzalin, wild type Arabidopsis roots grow normally, while 28-2b root tips swell and stop growing due to disruption of cell expansion and division. The roots of the 28-2b mutant are visibly indistinguishable from wild type in the absence of the drug oryzalin. We have mapped the 28-2b mutation to a 70 kb interval on the lower arm of chromosome three, and are working to identify the affected gene. The efforts to clone this gene as well as the implications of the 28-2b oryzalin supersensitive phenotype will be discussed.

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112 Making sense of the multitude of chloroplast protein import receptors in Arabidopsis thaliana

Bianca Hust[1], Andrea Voigt[2], Angelika Schierhorn[3], Mario Jakob[1], Ralf Bernd Kloesgen[1], Michael Gutensohn[1]

[1]Institute of Plant Physiology, Martin-Luther-University Halle-Wittenberg, Germany, [2]Plant Physiology, Humboldt University Berlin, Germany, [3]Department of Biochemistry, Martin-Luther-University Halle-Wittenberg, Germany

The majority of chloroplast proteins are encoded in the nucleus and therefore have to be imported into the organelle after their synthesis in the cytosol as precursor proteins carrying N-terminal transit peptides. The transport of precursor proteins across the outer and inner chloroplast envelope membrane is mediated by their interaction with two import machineries, the Toc and Tic complex, respectively. The core of the Toc complex essentially consists of two receptor proteins involved in the initial binding of precursor proteins at the chloroplast surface, Toc34 and Toc159, and the translocation pore, Toc75. In the genome of Arabidopsis thaliana two homologs of Toc34 (atToc33, atToc34) and four homologs of Toc159 (atToc159, atToc132, atToc120, atToc90) have been identified. For the functional characterization we have isolated knockout mutants for each of the six Arabidopsis Toc receptors. These mutants show remarkably different phenotypes suggesting more specialized functions for the import receptors. Examination of the proteome and gene expression profiles of the atToc33, atToc34, atToc159 and atToc132 mutants showed that in each case the accumulation of a specific subset of chloroplast proteins is affected. Further in vitro experiments using overexpressed receptor domains and specific antibodies suggest that each of these Toc receptors preferentially interacts with different groups of precursor proteins. Taken together the results indicate different substrate specificities of the chloroplast protein import receptors.

113 Functional analysis of AtSDK1 and AtSKD1-interacting proteins in the endosomal pathways of Arabidopsis

Thomas Haas, Marek Sliwinski, Marisa Otegui

Dept. of Botany, University of Wisconsin, 430 Lincoln Drive, Madison, WI 53706, USA

Endosomal compartments play a fundamental role in the sorting, recycling, and turnover of membrane proteins, the downregulation of receptors, and the trafficking of proteins to vacuoles and lysosomes in all eukaryotic cells. In addition, endosomal trafficking plays a essential role in various aspects of plant life, such as development, cell type specification, cell wall remodeling, gravitropic response, signal transduction, and hormone transport. Endosomal trafficking is regulated by a complex molecular machinery. Yeast Vps4p and mammalian SKD1 are AAA ATPases required for the endosomal sorting of secretory and endocytic cargo. We have identified a putative SKD1 homolog in Arabidopsis called AtSKD1. By immunogold labeling and expression of fluorescent fusion proteins, we have determined that SKD1 localizes to the cytoplasm and to multivesicular endosomes. In addition, GFP-SKD1 partially colocalizes with fuorescent fusion proteins of well characterized endosomal Rab GTPases such as AtRabF2b and RabF2a and with the endocytic markers FM4-64 and FM5-95. The expression of AtSKD1^{E232Q}, an ATPase-deficient version of AtSKD1, induces alterations in the vacuolar and endosomal systems of tobacco BY2 cells and ultimately leads to cell death. We have performed a yeast two-hybrid screening to identify AtSKD1-interacting proteins in *Arabidopsis*. One of the identified interactors, AtLIP5, is a homologue of mammalian LIP5/SBP1 and yeast Vta1p, which are known to be positive regulators of SKD1/Vps4p ATPase activity (Azmi et al. 2006 J. Cell Biol. 172, 705-717). We have isolated a knock out homozygous mutant line with a T-DNA insertion in *AtLIP5*. Although these mutant plants are viable, they exhibit reduced growth and produce fewer seeds. We are analyzing the subcellular localization patterns of AtSKD1 and AtLIP5 in the context of their putative interactions.

114 MscS-Like Proteins in Arabidopsis: Plastid Morphology and More

Elizabeth Haswell, Elliot Meyerowitz

Caltech

A fundamental question in biology is how cells perceive mechanical stimuli. Recent progress in the field of mechanotransduction has revealed that animal and bacterial cells sense sound, touch, and osmotic pressure through the action of mechanosensitive (MS) ion channels. Plants also respond to mechanical stimuli, and numerous mechanosensitive ion channel activities have been identified in plant membranes; however, the proteins responsible for these phenomena have not been identified. We have initiated the mechanistic and genetic characterization of a family of Arabidopsis proteins related to the bacterial mechanosensitive ion channel MscS, hypothesizing that they may be involved in mechanotransduction. Two Arabidopsis MscS-Like (MSL) proteins, MSL1 and MSL3, are capable of protecting bacteria from lysis upon hypoosmotic downshock, suggesting that at least some members of the family are mechanically gated ion channels. Results from subcellular localization and reporter gene experiments illustrate that the MSL protein family has evolved to function in the chloroplast and mitochondrial envelopes, as well as in other cellular membranes, and that family members are expressed in cells that undergo large, dynamic changes in turgor pressure. We have isolated insertional mutants in MSL2 and MSL3 and shown that msl2-1; msl3-1 double mutants have misshapen and enlarged plastids. Furthermore, MSL2- and MSL3-GFP fusion proteins are localized to the plastid envelope in discrete foci, and co-localized with the plastid division protein AtMinE (Haswell and Meyerowitz, Current Biology 16:1-11, 2006). The insertion in the msl3-1 mutant is predicted to produce a truncated protein that lacks the last 162 amino acids; we now find that GFP fused to a similarly truncated version of MSL3 is not localized to discrete foci but is evenly distributed around the plastid envelope. This finding suggests that localization to foci requires a specific domain in the C-terminus of MSL3, and that disruption of this domain may play a role in the plastid phenotype of the msl2-1; msl3-1 double mutant. Results from our ongoing characterization of the genetic and interactions between MSL2, MSL3 and previously identified plastid division genes will also be presented.

115 Molecular Genetic Analysis of Peroxisome Proliferation in Arabidopsis

Jianping Hu[1], Travis Orth[1], Sigrun Reumann[2], Jilian Fan[1], Sheng Quan[1], Xinchun Zhang[1], Mintu Desai[1], Navneet Kaur[1]

[1]MSU-DOE Plant Research Laboratory, Michigan State University, [2]Department of Plant Biochemistry, University of Goettingen

Peroxisomes are highly dynamic organelles with diverse functions in eukaryotes. In addition to beta-oxidation and H_2O_2 degradation, which are shared by most eukaryotes, plant peroxisomes play central roles in a variety of plant-specific processes, such as the glyoxylate cycle, photorespiration, nitrogen metabolism, hormone biosynthesis, and plant responses to abiotic and biotic stresses. Peroxisomes change their size, shape and number in response to various environmental stimuli to adapt to the new conditions. Although mechanisms controlling peroxisome abundance are still elusive, a number of yeast proteins have been identified to be involved with peroxisome proliferation with mostly unknown mechanisms. Plants lack obvious homologs to the majority of these yeast genes, suggesting that it is critical to reveal the plant-specific aspects of this fundamental cell biological process. As a starting point to identify components of the plant peroxisome proliferation machinery, we characterized the five-member Arabidopsis PEX11 protein family. We have shown that the PEX11 genes were amplified independently in the plant lineage after the split of different kingdoms, and that Arabidopsis and rice each contain two PEX11 subgroups. Using GFP-fusions and subcellular fractionations, we demonstrated the peroxisomal localization of all five PEX11 proteins and showed PEX11c and PEX11d to be integral membrane proteins. Overexpression studies suggested that different subfamilies of the Arabidopsis PEX11 family may play divergent roles during peroxisome proliferation. Consistent with this view, only a subset of AtPEX11 proteins was able to complement the yeast pex11 null mutant. Arabidopsis mutants created by virus-induced gene silencing and RNA interference are being analyzed. In addition, we performed genetic and biochemical screens to identify new peroxisome division/proliferation mutants and nuclear proteins that control the expression of key peroxisome proliferation genes. Mutants showing abnormal peroxisomal size or shape and a putative transcriptional factor that binds to the promoter of a light-inducible PEX11 gene have been isolated. Our research will help to establish a mechanistic model of plant peroxisome division and proliferation, which is currently lacking.

116 A Gain-of-Function Mutation in the Pleiotropic Drug Resistance Transporter AtPDR9 Confers Resistance to the Herbicide 2,4-D

Hironori Ito, William Gray

University of Minnesota

Arabidopsis contains 15 genes encoding members of the pleiotropic drug resistance (PDR) family of ABC transporters. These proteins have been speculated to be involved in the detoxification of xenobiotics, however little experimental support of this hypothesis has been obtained to date. Here we report our characterization of the Arabidopsis AtPDR9 gene. We isolated a semi-dominant, gain-of-function mutant, designated atpdr9-1, that exhibits increased tolerance to the auxinic herbicide 2,4-D. Reciprocally, loss-of-function mutations in AtPDR9 confer 2,4-D hypersensitivity. This altered auxin sensitivity defect of atpdr9 mutants is specific for 2,4-D and closely related compounds as these mutants respond normally to the endogenous auxins IAA and IBA. We demonstrate that 2,4-D, but not IAA, transport is affected by mutations in atpdr9 suggesting that the AtPDR9 transporter specifically effluxes 2,4-D out of plant cells without affecting endogenous auxin transport. The semi-dominant atpdr9-1 mutation affects an extremely highly conserved domain present in all known plant PDR transporters. The single amino acid change results in increased AtPDR9 abundance and provides a novel approach for elucidating the function of plant PDR proteins.

117 Interactions between the Microtubule plus end localising proteins EB1s and SPR1 in Arabidopsis

Despoina Kaloriti[1], David Rancour[2], Sebastian Bednarek[2], John Sedbrook[1]

[1]Department of Biological Sciences, Illinois State University, Normal, IL, 61790, USA, [2]Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, 53706-1544, USA

Microtubules (MTs) are involved in many processes of plant growth and development including cell expansion, cell division and organelle movement and secretion. The plant MTs form four basic arrays during the cell cycle: the cortical array, preprophase band, mitotic spindle and phragmoplast. Recent studies revealed that MTs in the cortical array exhibit dynamics at both ends. The plus end undergoes dynamic instability (periods of growth, pausing and shrinking), while the minus end undergoes slow depolymerisation (Shaw et al., 2003). In animals and fungi, dynamic instability is regulated by a collection of proteins called +TIPS (plus end tracking proteins) that are preferentially localized to MT plus ends. Little is known about what +TIPS exist in plants and how they regulate MT related processes. We have recently identified a novel, plant specific +TIP named SPIRAL (SPR1) in a forward genetics screen looking for Arabidopsis mutants affected in root cell expansion (Sedbrook et al., 2004). In addition to SPR1, Arabidopsis contains three +TIP proteins, which are homologues to the animal EB1 (End Binding) proteins. The function of EB1-like genes in plants has not yet been clarified, although GFP imaging studies of two of the three AtEB1 isoforms showed that they localise to MT plus ends with similar dynamics as seen with SPR1 (Chan et al., 2003; Mathur et al., 2003). We focused on identifying the relationship between SPR1 and EB1-like proteins in Arabidopsis. We phenotypically characterised plants with T-DNA insertional mutations in each of the three AtEB1 genes. Only one AtEB1 mutant exhibited a slight cell expansion defect related to root growth, while AtEB1 triple mutant roots exhibited the single AtEB1 mutant phenotype. Double mutant analysis was also performed between the AtEB1 mutants and spr1-6, with one AtEB1/spr1-6 double mutant exhibiting a severe root cell expansion defect, suggestive of a genetic interaction. In addition, all three AtEB1 homologues and SPR1-6 were tested in a Yeast Two Hybrid system to investigate possible protein-protein interactions. Our Yeast Two Hybrid results will be presented and discussed.

118 Trasngenic Expression of a Calcium-Binding Peptide: A Link Between ER Calcium Stores and Drought Tolerance?

Sang-Yoon Lee, Heike Winter-Sederoff, Dominique Robertson

Department of Botany, North Carolina State University

The development of osmotic stress tolerant crop plants is an important agricultural goal because of the increasing global demand for water, increased salinity in soil and declining aquifer reserves. Several calcium regulated protein kinases have been identified in *Arabidopsis* that affect osmotic stress tolerance. These kinases are regulated by transient cytosolic calcium oscillations that occur in response to a wide range of environmental stimuli. Cytosolic calcium concentration is maintained at a low level by Ca2+ transport proteins and the sequestering of higher calcium levels in intracellular compartments. We used a calcium binding peptide (CBP) derived from calreticulin, a calcium storage protein, to increase calcium levels in the endoplasmic reticulum (ER). The CBP was fused to the green fluorescent protein (GFP) and expressed in *Arabidopsis thaliana*. Compared to GFP vector control and wild type plants, the CBP plants showed increased root growth and better survival under osmotic stress conditions in 150 mM sorbitol medium, 75 mM and 150 mM NaCl media, and in soil. Analysis of gene expression demonstrated that CBP expression in the ER altered transcription of selected osmotic stress-inducible genes, including *CIPK6* (a serine/threonine protein kinase), *DREB1A*, *Dehydrin*, *rd29a*, and a *Myb* transcription factor. Some of these genes were upregulated in CBP transgenic plants in the absence of stress. These results identify a new link between ER calcium, root growth, and drought tolerance. Currently, we are investigating how ER CBP affects cytosolic calcium and whether constitutive expression of CIPK6 can increase drought tolerance.

119 CLB19, a Novel Pentatricopeptide Repeat Protein that Regulates the Expression of the Rubisco Large Subunit in Arabidopsis

Maricela Ramos[1], Arturo Guevara-Garcia[1], Charles Andres[2], Maria de la Luz Gutierrez-Nava[1], Ian Small[2], Patricia Leon[1]

[1]Instituto de Biotecnologia UNAM, [2]Unite de Recherche en Genomique Vegetale INRA

The biogenesis of chloroplasts is a complex process that is still not well understood. This differentiation occurs in response to specific signals in coordination to the differentiation of mesophyll and palisade cells. Thus chloroplast biogenesis depends on the coordinated expression of the nuclear- and chloroplast-encoded genes. In addition to various structural genes, the nucleus encodes for the majority of the regulatory proteins that modulate chloroplast gene expression. Most chloroplast transcripts are subject to extensive post-transcriptional processing and these events are essential for the expression of chloroplastic genes and for the correct organelle biogenesis. Today only few proteins involved in chloroplast transcript processing and translation are known. However, the identification of key elements required in this process represents not only a potential avenue for its future manipulation, but also has permitted to uncover novel biological processes.

To identify genes and factors required for proper development of a proplastid into chloroplast various mutants that block chloroplast development, at different stages during organelle differentiation (clb) were identified. One of these mutants severely affect chloroplast biogenesis, the clb19, is lethal and showed pale yellow seedling as it accumulates low levels of chlorophyll and carotenoid pigments. The molecular characterization of this mutant demonstrated that the gene responsible for its phenotype, CLB19, corresponds to a novel pentatricopeptide repeat (PPR) protein. This protein belongs to one of the largest gene families in plants, identified by a particular motif known as the pentatricopeptide repeat whose particular functions are basically unknown. The analysis of CLB19 has shown that this protein this precise function of this PPR protein has demonstrated its essential role in the post-transcriptional regulation during chloroplast development, that explains its dramatic phenotype. Our analysis has shown that CLB19 protein affects the expression of four chloroplast genes of the chloroplast genome. Our results demonstrate that at least one of the roles of CLB19 relates to the proper processing of both the atpE-atpB and rbcL transcripts. In particular, CLB19 is required for the efficient processing and translation of the catalytic subunit of the RbcL gene during a particular plant developmental stage. Together this data supports the essential role that post-transcriptional events play in the chloroplast biogenesis and exemplifies one of the most unique functions of this family of proteins.

120 Elucidation of the Molecular Role of SCD1 in Cytokinesis and Cell Elongation through the Identification of Binding Partners

Colleen McMichael[1], Tanya Falbel[2], Lisa Koch[1], Sebastian Bednarek[1]

[1]Department of Biochemistry, University of Wisconsin-Madison, [2]Department of Botany, University of Wisconsin-Madison

The timing of cell division, orientation of the division plane, and direction and extent of cell expansion are fundamental to plant morphology. Establishment of both the orientation of the division plane and direction of cell expansion are regulated by polarized membrane trafficking and cytoskeletal dynamics (1). Arabidopsis plants carrying mutations within the Stomatal Cytokinesis Defective 1 (SCD1) gene display defects in both cytokinesis and polar cell expansion, indicating that the SCD1 protein is critical to both processes (2). Based on the phenotypes of scd1 mutants and the conserved domains of the SCD1 protein, we predict that SCD1 plays a role in vesicular trafficking and/or cytoskeletal dynamics that is critical for cytokinesis and cell expansion. A combined genetic and biochemical approach is underway to define the molecular interactions of SCD1 in order to elucidate its function. Identification of the role of SCD1 will provide insight into the molecular mechanisms that link plant cytokinesis and cell expansion.

(1) Bednarek SY & Falbel TG. (2002) Membrane Trafficking During Plant Cytokinesis. Traffic 3: 621-629.

(2) Falbel TG, Koch LM, Nadeau JA, Segui-Simarro JM, Sack FD & Bednarek SY. (2003) SCD1 is required for cytokinesis and polarized cell expansion in Arabidopsis thaliana. Development 130: 4011-4024.

121 Characterization of the functions of AtCDC48 and the UBX-domain containing protein, PUX1

Sookhee Park, Dave Rancour, Sebastian Bednarek
University of Wisconsin-Madison

CDC48/p97 is an essential, AAA-ATPase chaperone that functions in numerous cellular activities. CDC48/p97 is recruited to specific functions through its interaction with adapter proteins. Analysis of loss of function and dominant negative Atcdc48 mutants demonstrates that the AtCDC48 is essential for plant growth and development. In particular, Atcdc48 mutants display defects in pollen transmission, embryo development and seedling growth. Homozygous Atcdc48 seedlings display gross morphological defects in the roots and shoots and arrest shortly after germination. To further understand the function of AtCDC48, we have sought to identify and characterize several putative AtCDC48 adaptors containing UBX domains. The Arabidopsis genome contains 15 UBX domain containing PUX proteins. Detailed analysis of PUX1 shows that it inactivates AtCDC48 ATPase activity through hexamer disassembly. To test the biochemical requirements for this disassembly process, a variety of AtCDC48 truncation and point mutants were generated to identify the specific domains and residues required for AtCDC48-PUX1 interaction. In addition, the effects of AtCDC48 nucleotide binding and hydrolysis mutations on the PUX1-mediated disassembly process were investigated. The sub-domain of AtCDC48 N-terminus (Na) is the primary binding site for PUX1. In Atcdc48 ATP binding and hydrolysis mutants, PUX1 binding was not affected indicating that binding is ATP independent. However, disassembly of the hexamer was influenced by the ATP binding and hydrolysis status of AtCDC48. This work provides mechanistic insight into the process of regulation of CDC48/p97 activity through PUX1-mediated AtCDC48 hexamer disassembly.

122 The Arabidopsis sku7 gene affects directional root growth and organ axial twisting

Chaithanyarani Parupalli, John Sedbrook
Illinois State University,Normal,IL-61790,USA.

Plants show differential cell expansion in response to external stimuli such as touch and gravity. These responses can be analyzed by growing *Arabidopsis* seedlings on 1.5% agar surface tilted at 45°. Under this condition, wild type roots exhibit a sinusoidal root waving pattern in a downward growth direction. In an effort to learn more about these processes, we isolated six *Arabidopsis* mutants (*sku6/spr1* through *sku11*), which exhibited abnormal root skewing patterns due to defects in cell expansion. Recent studies showed that *spr1* encodes a microtubule interacting protein involved in directional cell expansion (Sedbrook *et al*, 2004). The present study focuses on the phenotypic characterization and mapping of *sku7*, which has a similar root skewing pattern as that of *spr1*. We found that *sku7* roots exhibit right-handed axial twisting, while etiolated hypocotyls exhibit left-handed twisting. Unlike *spr1* and wild type, *sku7* root skewing is unaffected by propyzamide, an anti-microtubule drug. We have mapped the *sku7* mutation to a 400kb interval on chromosome 2 and are working to identify the affected gene. We also performed double mutant analysis between different *sku* mutants in order to find genetic interactions between them. The results showed that *sku7* is partially epistatic to *spr1* suggesting their possible role in a single pathway. *sku5/sku7* double mutant roots exhibited initial right skewing pattern followed by left skewing. Further characterization of microtubule organization in these double mutants, cloning and molecular characterization of the *sku7* gene should provide important insights into how cells expand in response to environmental stimulation.

123 The Arabidopsis CDC48 Adapter Protein PUX2 Plays a Role in ER Membrane Organization

David Rancour, Sookhee Park, Lisa Koch, Sebastian Bednarek

Biochemistry Department, University of Wisconsin-Madison, Madison, WI 53706

CDC48/p97 is a conserved and essential hexameric AAA-ATPase that functions as a molecular chaperone in numerous diverse cellular activities. CDC48/p97 activity is recruited to specific functions through its interaction with adapter proteins. Our working hypothesis is that AtCDC48 and the Arabidopsis homolog of syntaxin5/Sed5p, SYP31, mediates events important for plant cytokinesis. Using affinity chromatography and MALDI-TOF mass spectrometry we have identified two uncharacterized plant UBX-domain containing proteins, PUX1 and PUX2, which interact with AtCDC48. UBX-domains are ubiquitin-like protein folds that function as interaction domains for CDC48/p97. The Arabidopsis genome encodes 15 PUX proteins. All PUX proteins tested so far interact with AtCDC48 suggesting this to be an AtCDC48 regulatory protein family.

Characterization of PUX2 indicates that it is a peripheral membrane protein that interacts with AtCDC48 in vitro and co-fractionates with membrane-associated but not soluble AtCDC48 in vivo. Biochemical reconstitution and immunolocalization data suggest that PUX2 facilitates the interaction of SYP31 and AtCDC48 during interphase and cytokinesis, thereby regulating an AtCDC48 membrane-associated function. Deletion analysis of PUX2 protein domains reveals the requirement of its PUG domain and not its UBX-domain for interaction with AtCDC48. Sequence analysis of the UBX-domain from PUX2 suggests that divergence in amino acid composition might be responsible for the inability of the domain to interact with AtCDC48. Furthermore, analysis of protein domains of AtCDC48 required for interaction with PUX2 show that PUX2 binds AtCDC48 through the C-terminal D2 domain, an unconventional interaction for CDC48/p97 adapters. These studies provide the first evidence that the PUG domain may be an alternate interaction domain for AtCDC48. Loss-of-function pux2-1 Arabidopsis plants display altered endoplasmic reticulum (ER) distribution and a corresponding increase in cellular chloroplast content. These data taken together suggest a role for PUX2, AtCDC48 and SYP31 in maintaining ER membrane function. Models of PUX2 function will be presented.

124 Characterization of an Ectopic Cell Separation Mutant tfa1 'Things Fall Apart'

HONGYU RAO, Cory Hirsch, Joonyup Kim, Sara Patterson

Horticulture Department, University of Wisconsin at Madison

Although we often think of the plant cell wall as rigid and inflexible, it is dynamic, responding to developmental and environmental cues, and this plasticity is essential to basic plant development. Processes such as cell to cell adhesion and cell separation are examples of this dynamic nature of the plant cell wall; however, little has been studied about how plants integrate the processes of cell to cell adhesion and plant cell separation with developmental and environmental cues. We have identified several ectopic cell separation mutants that we have designated tfa "things fall apart." These mutants are all characterized by unregulated cell separation in epidermal, cortical and vascular tissues. This process is most severe in young seedlings affecting the hypocotyl, cotyledon, and first true leaves. Most of the young seedlings undergoing the irregular ectopic cell separation usually can not develop normal root and shoot system and eventually die when cultured on 1/2MS media with 0.8% agar. Crosses among the mutants indicate that two of these genes are allelic and recessive mutations, and we have designated them tfa1-1 and tfa1-2. Additional characterization of tfa1-2 has demonstrated that the defects in cell adhesion in tfa1-2 young seedlings can be partially avoided by culturing tfa1-2 seeds on the 1/2 MS media with a higher percentage agar or at low temperatures. Expression of several cell wall associated genes has been analyzed by RT-PCR and several of these genes show changes in expression in the mutant background. Microarray analysis of tfa1-2 has been conducted and there are numerous cell wall associated genes as well as others that are strongly upregulated or down regulated. tfa 1-2 was identified from an EMS screen of Arabidopsis seedlings and PCR-based mapping indicates that it is positioned on the right arm of Chromosome I. We will present phenotypic, physiological and molecular characterization of tfa1. We propose that there are several regulatory pathways affecting cell separation programs in the plant. Our studies on TFA1 will contribute to our understanding of how plants regulate cell to cell adhesion and cell separation processes during plant development.

This work was funded by USDA grant 0035301-9085 and NSF DBI

*Corresponding author: spatters@wisc.edu or hrao@wisc.edu

125 The Actin Depolymerizing Factor Gene Family in Arabidopsis: Expression Patterns and Cellular Localization

Daniel Ruzicka, Muthugapatti Kandasamy, Elizabeth McKinney, Brunilis Burgos, Richard Meagher
Department of Genetics, University of Georgia, Athens GA 30602

The actin-based cytoskeleton is comprised of actin and a variety of actin binding proteins (ABPs) that function coordinately to regulate cytoskeletal organization and remodeling. Actin Depolymerizing Factor (ADF) severs and depolymerizes actin filaments resulting in an increase in cytoskeletal dynamics, and has been implicated as the ABP responsible for transporting actin into the nucleus. In higher plants, ADF is encoded by a large gene family. The Arabidopsis ADF gene family contains eleven expressed genes grouped phylogenetically into three ancient subclasses. To account for the conservation of the three Arabidopsis ADF subclasses, we hypothesize there are essential differences in subclass expression patterns and protein isovariant functions.

The B-glucuronidase reporter gene was translationally fused to the promoter and enhancer intron of each ADF gene member to investigate subclass expression patterns. By including the putative enhancer intron, these GUS fusions more accurately and precisely represented each gene's expression pattern, showing agreement with data from Western blots using ADF monoclonal antibodies (mAb), cDNA amplification using RealTime-PCR, and microarray data from Genevestigator. Combining the phylogenetic subclass distinctions and individual gene expression pattern data, the Arabidopsis ADF subclasses exhibit distinct expression patterns including: 1. Constitutive, 2. Root or pollen, and 3. Rapidly differentiating tissue. Two recently diverged clades of subclass 2 ADF genes exhibit distinct pollen specific and root specific expression patterns, suggesting a recent divergence in the regulation of gene expression within this subclass.

To extend ADF localization to the cellular level, ADF subclass immunolocalization was performed using the mAb's developed against individual ADF proteins. Although immunolocalized ADFs only weakly decorated actin filaments, the three subclasses show distinct cellular localization patterns consistent with ADF's roles in remodeling actin filaments in the cytoplasm and transporting actin into the nucleus.

126 Determining the Chloroplast Division Roles of the Two *FtsZ* Families in *Arabidopsis thaliana*

Aaron Schmitz[3, 1], Bradley J.S.C. Olson[3, 2], David Yoder[3], Deena Kadirjan-Kalbach[3], Katherine Osteryoung[3]
[1]Cell & Molecular Biology Graduate Program, [2]Biochemistry & Molecular Biology Graduate Program, [3]Department of Plant Biology, Michigan State University, East Lansing, MI 48824

Plant chloroplasts, organelles of cyanobacterial origin, divide by fission to maintain proper numbers. The process of fission requires mid-plastid alignment of three rings: a Z-ring and an inner plastid division (PD) ring, both located within the stroma; and an outer PD ring located on the cytosolic side of the outer membrane. The Z-ring, composed of the tubulin-like protein FtsZ, is the initial ring to form during chloroplast division and is similar to the bacterial Z-ring, which serves as a scaffold for recruitment of other bacterial division components. Thus far, all plants investigated have two families of *FtsZ*, *FtsZ1* and *FtsZ2*. FtsZ1 and FtsZ2 differ primarily at their C-termini; FtsZ2, but not FtsZ1, contains a motif found in bacterial FtsZ proteins that is responsible for binding other cell division factors. The plant FtsZ2 C-terminal motif has been shown to be important for binding the chloroplast division protein ARC6. Recently, we determined that Arabidopsis maintains a 1:2 ratio of FtsZ1 to FtsZ2, and changes in FtsZ levels result in fewer enlarged plastids, presumably due to a block in chloroplast division. Interestingly, either increasing or decreasing the levels of individual FtsZ protein results in larger, fewer plastids; smaller and more numerous plastids have not been observed. We hypothesize that the ratio of FtsZ1 to FtsZ2 is critical for proper plastid division and that FtsZ1 and FtsZ2 differ functionally largely due to their divergent C-termini. To address these hypotheses, we are conducting experiments in which transgenes will be used to manipulate FtsZ1 and FtsZ2 levels and ratios in Arabidopsis FtsZ T-DNA insertion lines, and we are investigating the role of the divergent C-termini in FtsZ1 and FtsZ2.

127 AtCKT1, a Novel Transporter for Purines and Cytokinins in Arabidopsis thaliana

Benjamin Schumacher[2], Veronica Maurino[1], Esther Grube[1], Ulf Flugge[1], Marcelo Desimone[2]

[1]Botanical Institute, University of Cologne, Germany, [2]ZMBP, Plant Physiology, University of Tuebingen, Germany

Cytokinins (CK) allocation plays a key role in plant development. Both xylem and phloem serve for long distance transport of CK, but the mechanisms involved in their loading at the site of synthesis and unloading at the site of utilization remains unclear. A family of purine permeases (PUPs) and a family of nucleoside transporters (ENTs) are able to transport CK free bases and nucleosides respectively, suggesting that plants utilizes transporters with broad specificities for CK movement through cell membranes. In this work, a novel transporter of Arabidopsis was identified (AtCKT1). It belongs to a protein family with a member recently characterized in Aspergillus (AzgA1) and shows high homology to several proteins encoded in plant genomes. When expressed in a yeast mutant defective in adenine uptake (fcy2), AtCKT1 restored the capacity for adenine transport. Competition experiments indicate that CK are also potential substrates. Adenine uptake into yeast cells by AtCKT1 is coupled to proton transport as suggested by pH dependency and proton gradient inhibitors. Transient expression of GFP-AtCKT1 protein fusions in Arabidospis cells indicates localization of AtCKT1 in the plasma membrane. Transgenic plants carrying the reporter gene GUS under control of the AtCKT1 promoter and RT-PCR analysis revealed that AtCKT1 is mainly expressed in roots at relative low levels. T-DNA insertion lines producing knock out of AtCKT1 are resistance to toxic purine analogs and present lower sensitivity to CK application. In contrast, plants overexpressing AtCKT1 showed enhanced sensitivity to toxic purine analogs and CK, and increased capacity to adenine uptake from the environment. These data suggest that AtCKT1 is involved in purine and CK metabolism in Arabidopsis roots.

128 Genetic Analysis of SCY1 and SCY2 Function in Arabidopsis

Courtney Skalitzky, Jessica Harwood, Gregory Heck, Donna Fernandez

University of Wiscosin-Madison

SCY1 and SCY2 are plastid localized homologs of the bacterial SecY protein. The SecY translocon in the plasma membrane of eubacteria facilitates both the secretion of peptides out of the cell and the insertion of proteins into the lipid bilayer. In addition to one subunit of SecY, the translocon contains one subunit of SecE and SecG. Plastids contain SecY and SecE homologs, but not SecG. Two SecY homologs have been found in Arabidopsis, which we have designated as SCY1 (At2g18710) and SCY2 (At2g31530). Both SCY1 and SCY2 have ten predicted transmembrane domains and their amino acid sequences are only 28% identical and 44% similar. Previous studies showed SCY1 is targeted to the thylakoid membranes in the chloroplast. SCY2 sequence includes a putative transit peptide and import studies have shown that it is also targeted to the plastids. RT-PCR analysis revealed that SCY1 and SCY2 are expressed in both shoots and roots and both genes are constitutively expressed in the light and the dark. Despite these similarities, genetic analyses indicate that they play different roles in plant development. Mutations in *SCY1* cause a seedling lethal phenotype, while mutations in *SCY2* result in embryo lethality. To investigate whether expression differences account for functional differences, we performed promoter swap experiments. Based on our current results, we conclude that differences in function are more likely to form the basis of the different mutant phenotypes than expression differences.

One possibility that we are considering is that SCY1 and SCY2 function in different membranes within plastids. We are using SCY2-GFP protein fusions to determine the subcellular localization of SCY2. Supported by UW-Madison Graduate School.

129 The *Arabidopsis elch* mutant reveals a link between an ESCRT-like pathway and cytokinesis

Christoph Spitzer, Swen Schellmann, Aneta Sabovljevic, Mojgan Shahriari, Channa Keshavaiah, Martin Hulskamp
Botanical Institute III, University of Cologne, Germany

Few years ago an alternative route to the proteasomal protein degradation pathway was discovered that specifically targets proteins marked with a single ubiquitin to the endosomal multi-vesicular-body (MVB) and subsequently to the vacuole (yeast)/lysosome (animals) where they are degraded by proteases. Vps23 and TSG101 respectively are key components that recognize monoubiquitinated proteins and sort them through the ESCRT I-III complexes into the multivesicular-body (MVB). After fusion of the MVB to the vacuole/lysosome the internal vesicles are accessible to luminal proteases.

Here we report that the Arabidopsis ELCH gene encodes a Vps23/TSG101 homolog. ELCH binds monoubiquitin in vitro and interacts in yeast two hybrid analysis with the putative plant homolog of Vps37 which is part of yeast and mammalian ESCRT I complex. Whereas studies in yeast and mammals have focused mainly on sorting defects of plasma membrane proteins we found that ELCH is important during cytokinesis in Arabidopsis. Cell division in plants depends on vesicular transport, rearrangement of the endomembrane system and microtubule arrays. Accordingly we found that ELCH interacts genetically with the kinesin like protein ZWICHEL/KCBP that has been shown to localise to microtubule arrays like preprophase band and mitotic spindle in dividing cells. Compromising microtubule dynamics in elch mutant background leads to a drastic enhancement of the otherwise subtle phenotype. In summary our data suggests that an ESCRT-like pathway is conserved in Arabidopsis and plays an important role during cytokinesis.

This work is supported by SFB635.

130 Expression Patterns, Mutagenesis, and Protein Interactions of Arabidopsis thaliana DRG Interaction Protein-2 (DRI-2)

Jennifer Kubic, Joel Stafstrom
Northern Illinois University, Department of Biological Sciences, DeKalb, IL 60115

GTP binding proteins (G protein) are vital to many cellular processes including signal transduction (Ras), translation (eIF2, EF-Tu), cellular trafficking (ARF, SAR), cytoskeleton construction (Rho, Rop), and nuclear translocation (Ran). G proteins are capable of acting as molecular switches in these processes: when they bind to GTP they are in the active conformation; upon hydrolysis GTP and binding to GDP, they become inactive. The Developmentally Regulated GTP binding protein (DRG) family is highly conserved. Amino acid identity among fungal, animal and plant DRG1s is about 71%, and AA identity of DRG2s is nearly as high. Arabidopsis DRG1 (At4g39520) and DRG2 (At1g17470) are about 57% identical. Arabidopsis DRG3 (At1g72660) is 95% identical to DRG2, suggesting a recent gene duplication. The function of the DRGs remains unknown. Nevertheless, this high level of conservation and the important roles played by other G proteins suggests an important physiological function for DRGs as well. The DRGs are closely related to a family of bacterial G proteins called OBGs. Since OBGs are involved in ribosomal function and DNA replication, similar activities may be regulated by DRGs. Proteins that interact are often involved in the same pathway. Based on global yeast interaction experiments, both DRGs interact with the same protein, YDR152w. The apparent homologue of this gene in Arabidopsis thaliana (At1g51730) is called DRG Interacting protein-2 (DRI-2). Analysis of expression patterns, mutagenesis, and protein interaction partners of AtDRI-2 may provide clues to the function of an important family of G proteins, the DRGs.

131 Sub-Cellular Localization of DRG Family Protein

Benjamin Nelson, Joel Stafstrom

Department of Biological Sciences, Northern Illinois University, Dekalb, IL 60115

DRGs (Developmentally Regulated G-Proteins) were first discovered in embryonic mouse brains. All eukaryotes appear to have two *Drg* genes (Drg1 and Drg2), Archaea have a single gene, and bacteria have one Obg gene, which encodes a related G-protein. DRG proteins are very highly conserved. Amino acid identity among fungal, animal and plant DRG1s is about 71%, and AA identity of DRG2s is nearly as high. Arabidopsis DRG1 (At4g39520) and DRG2 (At1g17470) are about 57% identical. Arabidopsis DRG3 (At1g72660) is 95% identical to DRG2, suggesting a recent gene duplication. This level of conservation suggests that these proteins perform an important cellular activity, yet relatively little is known about the function of these proteins. Single-gene knock-outs of these genes, produced both by T-DNA insertion and RNA interference, appear quite normal when grown under normal conditions. Double mutants are being generated. Growth and development under a variety of stress conditions is being analyzed as well. A variety of techniques are being used to determine patterns of subcellular localization, including standard biochemical fractionation, affinity pull-down assays, and GFP fusions in transgenic plants. Differential and rate-zonal centrifugation demonstrates that DRG1 and DRG2 co-localize with various ribosome populations. However, the specific localization patterns are unique, suggesting a distinct function for each protein. We have also found that the full-length 45kd DRG2 protein is processed to 43kd and 30kd forms, each of which is found in specific subcellular fractions. Affinity pull-down assays using FLAG-tagged ribosomes, in both Arabidopsis and Saccharomyces, also indicate that the DRG proteins are ribosome-associated. GFP studies, while in the early stages, indicate a cytosolic and possibly nuclear localization for the DRG proteins.

132 A Mutation in Translocon of Outer Membrane of Chloroplasts 132 (Toc132) Enhances the Gravitropic Defect of the altered response to gravity 1 (arg1) Mutant

John Stanga[1], Kanok Boonsirichai[2], John Sedbrook[3], Patrick Masson[1]

[1]University of Wisconsin-Madison, [2]Center for Atomic Research for Peace Purposes, Bangkok, Thailand , [3]Illinois State University, Normal, Illinois

Arabidopsis roots perceive gravity and reorient their growth accordingly. The root cap is necessary for a full response to gravity. Starch-dense amyloplasts within the columella cells of the root cap are important for gravitropism, as starchless mutants (pgm1) display an attenuated response to gravistimulation. However, our understanding of the molecular events controlling this behavior is incomplete. The altered response to gravity 1 (arg1) mutant is involved with the early phases of gravity signal transduction and is in a genetically distinct pathway from pgm1. arg1 roots and hypocotyls respond slowly to gravistimulation. Expression of ARG1 in the root cap is sufficient to rescue the root gravitropism defect specifically, while expression of ARG1 in the endodermis is sufficient to rescue the hypocotyl defect specifically. arg1 seeds were mutagenized with EMS to identify new mutants that would enhance the gravitropic defect of arg1 and therefore be potential members of the PGM1 pathway. The roots of one such mutant, mar2, grow in random directions only when arg1 is present, yet have no other obvious defects. Also, mar2 amyloplasts appear normal when observed by electron microscopy. mar2 plants possess a mutation in the Translocon of Outer Membrane of Chloroplast 132 (TOC132) gene, At2g16640, that results in premature termination of translation. Overexpression of TOC132 rescues the random growth phenotype of arg1mar2 roots. In addition to green tissues, Toc132 is expressed in tissues that lack chloroplasts, such as the columella cells of the root cap. Toc132 is thought to act as a receptor component of the Toc complex. Its exact contribution to the determination of root growth direction remains the subject of ongoing endeavors.

133 The Road Less Travelled: Investigating The Multi-Step Targeting Pathway of Tic40

Joanna Tripp, Kenneth Keegstra, John Froehlich

DOE-MSU Plant Research Laboratory, Michigan State University, East Lansing MI, 48823 (USA)

Proteins destined for the chloroplastic inner envelope membrane appear to use the general import pathway, but then get diverted to the inner envelope membrane by an unknown process. The pathways and mechanisms for targeting proteins to the chloroplastic inner envelope membrane are poorly characterized. Based on analogy with mitochondria, where targeting to the inner membrane has been extensively studied, two hypotheses are possible. First is the stop transfer hypothesis whereby transport across the inner envelope membrane is halted, leading to insertion into the inner envelope membrane during the import process. The second hypothesis, sometimes called the conservative sorting hypothesis, predicts that import into the stroma is completed before insertion into the inner envelope membrane occurs as a discrete second step.

Tic40 is part of the protein import apparatus located at the inner envelope membrane of chloroplasts. It is anchored in the inner envelope membrane by a single N-terminal transmembrane domain and has a topology in which the bulk of the C-terminal domain is orientated toward the stroma. In vitro studies indicate that the targeting of Tic40 to the inner envelope membrane involves two steps. Using an in vitro import assay, we have shown that in the first step, Tic40 is initially transported across the inner envelope membrane into the stroma. The resulting Tic40 stromal intermediate is subsequently retargeted to the inner envelope membrane by an unknown mechanism. The sorting of Tic40 requires a bipartite transit peptide, which is first cleaved by the stromal processing peptidase (SPP) thus generating a soluble Tic40 stromal intermediate (iTic40). iTic40 is further processed by a second peptidase, possibly a Type I Signal peptidase, which generates its mature form (mTic40). Using deletion mutants, we are investigating and characterizing the targeting determinants of Tic40 involved in this multi-step targeting pathway. Presently, we have identified a sequence motif N-terminal of the transmembrane domain which appears to prevent arrest of Tic40 at the inner envelope membrane and allows complete transport of Tic40 into the stroma. Likewise, we are continuing to investigate and identify additional targeting determinants that are required for reinsertion of iTic40 into the inner envelope membrane. Finally, by examining the multi-step insertion of Tic40 into the inner envelope, we envision that Tic40 may be a good 'model' protein that will allows us to investigate in greater detail the 'conservative sorting pathway' within chloroplasts.

134 *Arabidopsis* microtubule-associated protein SPIRAL2 affects microtubule dynamics

Maki Yao, Tsubasa Shoji, Takashi Hashimoto

Nara Institute of Science and Technology(NAIST), Nara, Japan

In plant cells, various **microtubule-associated proteins (MAPs)** regulate many **microtubule (MT)** functions such as ordered array formation in interphase and phragmoplast development. However, exact biochemical functions of MAPs are largely unknown. *Arabidopsis* **SPIRAL2/TORTIFOLIA (SPR2)** protein is a plant-specific MAP involved in the regulation of cortical MT arrays which dictate the direction of cell expansion. SPR2 localizes to MTs in vivo, and the null mutation of SPR2 causes a shallow left-handed helical array in cortical MTs and a right-handed helical growth in longitudinally growing organs such as hypocotyls, petioles and flowers. SPR2 has HEAT repeat motifs at the N-terminal region and highly conserved region among SPR2 homologs at the C-terminus. In MT binding assays in vitro, non-overlapping N- and C-terminal regions of recombinant SPR2 directly bound to taxol-stabilized MTs. We also examined the effect of SPR2 on the dynamics of MT by dark-field microscopy using the recombinant proteins of full-length and N-terminal SPR2. Results will be presented.

135 Discovering the function of WAVE-ARP2/3-generated actin filaments during plant cell morphogenesis

Chunhua Zhang[1], Taisiya Zakharova[1], Jie Le[1], Eileen Mallery[1], Salah El-Din El-Assal[2], Daniel Szymanski[1]
[1]Purdue University, [2]Cairo University

The leaf epidermis is a critical organ that regulates gas-exchange and water loss, as well as being the first line of resistance against insect and pathogen attack. Epidermal cell functions are intimately tied to their complex shapes. For example, the highly polarized pavement cells have a complex lobed structure that allows adjacent pavement cells to interlock and tightly seal internal compartments from the external environment. Trichomes are branched, highly polarized cells that protect the plant against insect attack. We are using both cell types as models to understand how the actin and microtubule cytoskeletons are coordinated during epidermal morphogenesis. Actin Related Protein (ARP) 2/3 is a heteromeric 7 subunit complex that efficiently nucleates actin filaments. In plants ARP2/3 is required for polarized trichome growth, as well as for the normal development of pavement cell shape and cell-cell adhesion. ARP2/3 requires positive regulation by another heteromeric complex termed WAVE (Szymanski, 2005). Although Arabidopsis continues to provide important information regarding the in vivo function of individual WAVE and ARP2/3 complex proteins (Djakovic et al., 2006; Le et al., 2006), little is known about the cellular function of these important complexes and the actin filaments that they generate. The combination of well characterized mutants and new cytological tools has allowed us to identify the active pools of WAVE and ARP2/3 in growing epidermal cells. Cell fractionation, localization data, and live cell imaging assays indicate that ARP2/3 functions are intimately linked to the positioning and biogenesis of the centeral vacuole. We will present our data indicating that plants cells have multiple and unique uses for the evolutionarily conserved WAVE and ARP2/3 complexes.

Szymanski (2005) Current Opinion Plant Biol. 8(1): 103-12
Dajakovic et al. (2006) Development 133: 1091-1100
Le et al. (2006) Current Biology 16:1-7

136 MIKC* MADS-box transcription factors contribute to late pollen development in Arabidopsis

Benjamin Adamczyk, Donna Fernandez
University of Wisconsin-Madison, Madison, WI

The MIKC* clade of MADS-box genes in Arabidopsis consists of six members, whose function has not been previously defined. Members of the MIKC* clade are structurally similar to the well-characterized MIKC^c MADS-box factors, but have a less-conserved keratin-like domain and a longer intervening domain. Our lab has shown that all six MIKC* genes are expressed in developing embryos (Lehti-Shiu et al. (2005) Plant Mol. Biol. 58: 89-107), and other sources have shown that they are prominently expressed in mature pollen as well. To determine whether MIKC* factors contribute to embryo or pollen function, we analyzed homozygous mutant plants for embryo and pollen defects. No developmental changes were seen in the single mutants. Since functional redundancy is common with MADS-box factors, we also generated double, triple, and quadruple mutant combinations. While embryo development was not affected in any of the mutant combinations, we found that pollen function was compromised. Reciprocal crosses revealed that three MIKC* factors play a redundant gametophyte-specific regulatory role in pollen development. Triple mutant pollen cannot compete effectively with wild type or double mutant pollen, resulting in reduced transmission of the triple mutant allele combination. We recovered plants homozygous for all three mutant alleles, which are viable but show fertility defects. In vitro assays have shown that the rate of mutant pollen germination is reduced relative to wild type, and pollen tube growth may also be defective. We are currently carrying out pollen competition assays with additional double, triple, and quadruple mutant combinations to determine whether other pollen-expressing MADS-box factors contribute to pollen function. Supported by USDA NRICGP (2001-35304-10887), and the UW-Madison Graduate School.

137 Importance Of Seed Biotin Protein In Development Of Arabidopsis Thaliana Seeds

Libuse Brachova[1], Hilal Ilarslan[2], Eve Syrkin Wurtele[2], Basil Nikolau[1]

[1]Iowa State University, Department of Biochemistry, Biophysics and Molecular Biology, Ames, IA, [2]Iowa State University, Department of Genetics, Development, & Cell Biology, Ames, IA

Plants are a major source of biotin in the biosphere. The biochemical function of biotin as an enzyme cofactor is to catalyze carboxylation, decarboxylation and transcarboxylation reactions. Furthermore, recent studies indicate that biotin has a new function as a regulatory molecule, controlling gene transcription. Uniquely to plants, biotin appears to be stored in seeds, covalently bound to a novel protein that hyper-accumulates in seeds. This Seed-Biotin Protein (SBP) has been found in carrots, pea, soybean and Arabidopsis. To understand the physiological role of the SBP-bound biotin, we have started to genetically characterize the function of this protein in Arabidopsis thaliana. We have identified two independent mutant lines, in which the SBP-coding gene (At2g42560) is disrupted with T-DNA and SpM-transposon insertions, respectively. Physiological, biochemical, and histological characterizations of these mutant lines indicate that the biotin associated with the SPB protein is required for the timely establishment of the Arabidopsis seedling. The phenotype associated with the loss of SBP function, can be reversed by the exogenous supply of biotin, indicating that SBP does in fact act as a biotin-store that is required for timely establishment of seedlings.

138 Mutation of the MAP Kinase Gene MPK6 Reduces Male Fertility in Arabidopsis

Susan Bush, Suraphon Chaiwongsar, Patrick Krysan

Horticulture Department and Genome Center of Wisconsin, University of Wisconsin-Madison, Madison, WI 53706, USA

The Arabidopsis mitogen-activated protein kinase (MAPK) MPK6 is activated in response to a variety of stressors, including low temperature, touch, wounding, salt, osmotic stress, pathogen attack, and ozone exposure. As part of a comprehensive analysis of MAPK function in Arabidopsis, we are characterizing two independent T-DNA alleles of MPK6. Our analysis has indicated that mutation of MPK6 causes low seed set that is likely to be due to defects in pollen development and/or maturation. Analysis of mpk6 pollen by environmental scanning electron microscopy has revealed mutant pollen to be less abundant and poorly dehisced from the anther compared to the wild-type. We are in the process of completing a study of pollen development in mpk6 anthers based on the use of thin-section microscopy, which should allow us to identify the stage of development at which mpk6 pollen become compromised, as well as the precise characteristics of any developmental irregularities. Genevestigator, the Arabidopsis database of Affymetrix gene chip data, indicates that the highest level of MPK6 expression occurs in the stamen and pollen, which is consistent with our observations of reduced male fertility in mpk6 plants. We are currently characterizing the expression pattern of MPK6 using GUS- and YFP-MPK6 fusion proteins in order to more precisely map MPK6 expression during floral development.

139 WRINKLED1, an AP2/EREBP Class Transcription Factor, Plays a Vital Role in Seed Oil Accumulation, Seed Germination and Seedling Establishment

Alex Cernac, Christoph Benning
MIchigan State University

The regulation and dynamics of seed oil accumulation and storage reserve utilization is a topic of economic and scientific interest. Through density screening and visual scoring the wrinkled1 mutant of Arabidopsis was isolated. The primary phenotype of wri1 is a defect in seed oil accumulation (80% TAG reduction wri1). Physiologically, the major wrinkled1 defect in the developing embryo is in the activities of five glycolytic enzymes. Hexokinase and pyrophosphate dependent phosphofructokinase activities were the most severely affected followed by pyruvate kinase and enolase activities, with aldolase activity showing a minor reduction. Taken together the data support a global role for WRI1 in the regulation of glycolysis. It was subsequently shown that WRI1 encodes an AP2/EREB domain protein and microarray data have demonstrated the wri1 mutant results in a down regulation in the accumulation of transcript for a large number of genes involved in carbon metabolism in the developing embryo. Examination of germination and gene expression indicates that WRI1 plays a role in abscisic acid response in the germinating seed, possibly as a result of altered storage reserve levels in the seed or altered metabolism which in turn affects abscisic acid response through sugar signaling. Our recent elucidation of the DNA binding motif for WRI1 has allowed us to focus research efforts on the targets of WRI1 activity and further define the roles of WRI1 in the developing embryo and germinating seed.

140 A bHLH Protein Involved in Embryo Pattern Formation Interacts With DORNROESCHEN (DRN) and DRN-LIKE and Reveals a Role for All Three Proteins in Seed Germination

John Chandler, Wolfgang Werr
Department of Developmental Biology, University of Cologne, Gyrhofstrasse 17, D-50923 Cologne, Germany

DRN and DRN-LIKE are two gene paralogues expressed from early embryo stages which redundantly control embryo patterning. A gene encoding a basic helix-loop-helix (bHLH) protein was isolated from a yeast two hybrid screen using the AP2 domain from DRN and DRN-LIKE as a bait. The protein-protein interactions were confirmed via both transient bi-molecular fluourescence complementation in leek cells and by co-immunoprecipitation experiments. The expression pattern of bHLH overlaps with DRN and DRN-LIKE in the early embryo and two independent mutant bhlh alleles show more severe defects in embryo development than in drn and drn-like single mutants, showing that the bHLH gene has an essential function in pattern formation. Double mutants between bhlh and drn and drn-like reveal an additional novel delayed seed germination phenotype not seen in any of the single mutants, which can be partially overcome by gibberellin. Taken together, our results show that this bHLH gene is a novel player in the complex network of regulatory genes controlling embryonic pattern formation and implicates novel heterodimers involving at least two transcription factor families in the control of seed germination.

141 *HAESA* and *HAESA-like 2* Activate Floral Organ Abscission In An Ethylene-Independent Manner

Sung Ki Cho, David Chevalier, Kevin Lease, Jason Doke, John Walker
University of Missouri

Organ abscission involves the regulated separation of cell layers to cause detachment of an organ from a plant. Our goal is to understand the molecular basis of this process. HAE and HSL2 exhibit similar promoter::GUS expression at floral abscission zones. Single knockout mutant lines of either gene do not show any phenotype, but the hae hsl2 double knockout mutant show an abscission-defect phenotype. The abscission defect observed in the double mutant could either be due to the inability to differentiate the abscission zone, or an inability to activate the cell separation process. Longitudinal sections through the abscission zone were examined by light microscopy and the fracture planes at the abscission zone when petals were forcibly removed were examined by scanning electron microscopy. These observations showed that the abscission zone in the hae hsl2 double mutant appears structurally normal. Furthermore, the hae hsl2 petal breakstrength at all flower positions was similar to that of wild type flowers that have not yet begun to abscise their petals. Taken together, these data support the idea that the role of HAE and HSL2 is to activate cell separation, rather than promote the differentiation of the abscission zone. Ethylene is also known to promote abscission; therefore we tested the ethylene-induced triple response and the effect of exogenous treatment on floral organ in the double mutant, revealing that HAE and HSL2 act independently of ethylene. We hypothesize that HAE and HSL2 activate abscission in an ethylene-independent manner by inducing the expression of enzymes that promote cell separation.

142 The Arabidopsis EARLY IN SHORT DAYS 7 (ESD7) Locus Encodes The Catalytic Subunit of DNA POLYMERASEε

Ivan Del Olmo[1], Mar Martin-Trillo[2], Jose Martinez-Zapater[2], Manuel Pineiro[1], Jose Jarillo[1]
[1]Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Departamento de Biotecnologia, 28040 Madrid, Spain., [2]Centro Nacional de Biotecnologia; Departamento de Genetica Molecular de Plantas, Cantoblanco, Madrid 28049, Spain.

The suitable control of the floral transition is crucial for reproductive success in flowering plants. In the last years, several early-flowering mutants have been characterized although much of their interactions with the inductive pathways of flowering are not known. In a collection of Ds-containing T-DNA lines in Ler background, we have isolated the *esd7* mutation, which causes early flowering independently of photoperiod conditions. Because the Ds element did not cosegregate with the early flowering phenotype, the ESD7 locus has been identified by a map-based cloning approach. ESD7 encodes the catalytic subunit of DNA polymerase ε (AtPOL2A), which is essential for the correct development and viability of the embryo (Ronceret et al., 2005, The Plant Journal 44:223-236). The esd7-1 mutation is affecting a conserved glycine residue boundering the C1 region of the protein. RT-PCR expression analysis for ESD7/AtPOL2A shows a very low expression level in most of the analyzed tissues; however, this expression is markedly increased by exposure to genotoxic agents; a 1 Kb promoter fusion AtPOL2A:GUS reveals that this gene is mainly expressed in the shoot and the root apical meristems and the vascular tissues at the seedling stage. The mutant phenotype is quite pleiotropic resulting in plants smaller in size and less vigorous than wild-type plants; microscopic analyses show that the epidermal cell size of mutant leaves is increased in comparison to wild type, although the cell number is smaller. Besides, the esd7-1 mutant shows narrowed leaves and alterations in the pattern of vegetative growth, mainly a reduction of the adult vegetative phase; in addition, the development of the root is also affected in the esd7-1 mutant, as it has been described for another hypomorphic allele of this gene (Jenik et al., 2005, The Plant Cell 8:3362-3377); the primary root of the mutant displays a significant decrease in elongation that is accompanied by a higher production of adventitious roots. Genetic analysis between esd7-1 and mutants affected in flowering inductive pathways suggests that flowering inhibition mediated by ESD7 may occur through different pathways and that is totally dependent on FT and SOC1 expression. Expression analysis of the floral integrators FT and SOC1 and the flowering time genes such as FLC and CO in esd7-1 mutant will be presented.

143 The Ubiquitin-Specific Proteases of *Arabidopsis thaliana*

Jed Doelling[1], *Allison Phillips*[2], *Gulsum Soyler-Ogretim*[1], *Richard Vierstra*[2]

[1]Division of Plant and Soil Sciences & The Genetics and Developmental Biology Program, West Virginia University, Morgantown, WV 26506, [2]Department of Genetics, University of Wisconsin-Madison, Madison, WI 53706

The ubiquitin/26S proteasome pathway is a major pathway of selective protein degradation in eukaryotic organisms. One class of enzymes essential to the function of this pathway is that composed of the ubiquitin-specific proteases (UBPs). The UBPs serve to detach ubiquitin monomers 1) from the primary translation products of the polyubiquitin and ubiquitin extension protein genes, 2) from multi-ubiquitin chains joined together via isopeptide linkages, and 3) from ubiquitinated proteins. We are investigating the specific roles performed by particular UBPs in *Arabidopsis thaliana* using a reverse genetic approach. We are currently analyzing a collection of T-DNA insertion lines that includes one or more mutant lines for 26 of the 27 UBP genes found in Arabidopsis. In all of these lines, the insertion site is located between the translational start and stop codons suggesting that homozygous individuals would be deficient in the function of the corresponding protein. No aberrant phenotypes were observed for many of the single gene, homozygous mutant individuals suggesting that the corresponding proteins are not essential or that other proteins may perform overlapping functions. Double and triple mutant lines are being assembled via genetic crosses to investigate the functions of UBP subfamilies. Particular UBP subfamilies appear to be essential for proper pollen development and function, for completion of embryo development, and for proper development and/or function of the female gametophyte.

144 Characterization of Organ Patterning During Fruit Development in *Arabidopsis thaliana*

Amanda Durbak, Erica Hsiao, Frans Tax

University of Arizona, Tucson, AZ, USA

While the fruit of a flowering plant plays an essential role in species propagation by housing developing seeds and aiding in their dispersal, the mechanisms that underlie intercellular signaling in patterning of fruit organ number remain unknown. Work in tomato has shown that increase in both organ number and cell division are responsible for larger fruit, but the molecular mechanism through which these two components are integrated and contribute to overall patterning and size is still not clear. While some mutants with organ number defects such as the clavata mutants have been identified in Arabidopsis, a mechanism of generation of extra organs has not yet been elucidated. In order to identify new mutants that have fruit patterning defects, we have implemented an EMS mutagenesis screen to look for plants that produce siliques with extra valves and margins. From this screen we have identified two new clv1 or clv2 alleles and two mutants that map to regions in the genome that are not known to contain genes involved in fruit patterning. Further characterization of the newly identified mutants along with previously identified mutants utilizing cell-type specific markers is being used to analyze changes in morphology, specification, and cell proliferation in mutant fruit. These studies will help to determine the key differentiation events responsible for fruit patterning.

145 Cryptochrome 2 (cry2) in Vascular Bundles Regulates Flowering in Arabidopsis

Motomu Endo, Nobuyoshi Mochizuki, Tomomi Suzuki, Akira Nagatani

Laboratory of Plant Physiology, Graduate School of Science, Kyoto University, Kyoto, Japan

Light is one of the most important stimuli to trigger the transition from vegetative phase to reproductive phase, namely flowering. In Arabidopsis, phytochrome B (phyB) and cryptochrome 2 (cry2) are major photoreceptors to regulate flowering time. Both are known to be expressed in almost all organs/tissues. It has been well-known since 1937 that plants perceive the light stimuli only in leaves to regulate flowering. Hence, photoreceptors in restricted parts can regulate flowering. We have investigated this issue and demonstrated that phyB regulates flowering time only in mesophyll cells (Endo et al., 2005). However it had remained unclear where cry2 regulates flowering.

Here, we expressed a cry2-GFP fusion protein in the *cry2* mutant background in organ/tissue specific manners by using specific promoters. We confirmed that cry2-GFP was expressed in expected places for all the promoters. Immunoblot, GFP fluorescence and real time PCR analyses demonstrated that the expression levels in respective organs/tissues in these lines were comparable to those of the authentic cry2. Transgenic lines expressing cry2-GFP in vascular bundles exhibited full complementation of the late flowering phenotype. By contrast, the other lines that expressed cry2-GFP in mesophyll, epidermis, SAM or root flowered late. *FT* is a key positive regulator of flowering. It is expressed in vascular bundles. We examined *FT* regulation by cry2 at the tissue level. In the lines expressing cry2-GFP in vascular bundles, *FT* mRNA was increased in vascular bundles. As expected, no induction of the *FT* expression was observed in other lines. Hence, only cry2 in vascular bundle regulates *FT* expression, which takes place in vascular bundles.

146 Analysis ff Natural Genetic Variation in Photoperiodic Regulation of Flowering Time in *Arabidopsis Thaliana*

Antonis Giakountis, Frederic Cremer, George Coupland

Max Planck Institute For Plant Breeding Research, Carl-von-Linne weg 10, D-50829 Cologne, Germany

Plants utilize a variety of endogenous signals and external stimuli to regulate the transition from vegetative growth to flowering. In *Arabidopsis, GIGANTEA* (*GI*) a clock output gene expressed in the evening, is considered to mediate between the circadian clock and transcription of *CONSTANS* (*CO*), another major gene of the photoperiod pathway. Upon exposure to inductive long-day conditions *CO* expression is up-regulated and in the presence of blue and far-red light, the stabilized CO protein activates the expression of the downstream floral integrator *FT*.

The effect of photoperiod on flowering-time control of *Arabidopsis* has been studied by analyzing flowering under extreme photoperiods of 8-10 h and 16 h, however under natural conditions photoperiod varies continuously with the changing seasons. We have described the flowering-time behaviour of model accessions such as Columbia and Ler at 6 different daylengths. Natural variation between accessions has been widely used as a tool for functional genetics, allowing the identification of genetic and/or allelic variation for a trait of interest. We have used two collections of *Arabidopsis* accessions in order to identify natural variation in their flowering time responses under a wide range of photoperiods. In addition forty transgenic lines of flowering time genes have been included in the analysis and their photoperiod responses are compared with those of the accessions. In order to evaluate the contribution of the circadian clock in photoperiod discrimination, the same accessions were transformed with a promoter fusion of *GI* to the firefly luciferase gene.

A two way ANOVA approach revealed the presence of extensive natural variation in the flowering time responses of accessions under different photoperiods. By performing a real time monitoring of the bioluminescence under the same range of photoperiods as for flowering time, we were able to identify additional natural variation in the peak time of *GI* expression under different daylengths. A selection of interesting accessions was made and expression analysis of several genes of the photoperiod pathway together with QTL mapping are in progress in an attempt to identify the sources of the observed natural variation at the molecular level.

147 Systemic signal transduction of flowering and protein trafficking of FT

Megumi Takahashi, Eiko Himi, Koji Goto
Research Inst. Bio Scineces, Okayama

Arabidopsis TERMINAL FLOWER 1 (TFL1) and FLOWERING LOCUS T (FT) are homologous proteins, that is 54% of amino residues are conserved in the whole region. However, the developmental role of these proteins are opposite; while tfl1 mutants show early flowering, ft mutants show late flowering. In addition to the early flowering phenotype, tfl1 shows terminal flower, suggesting that TFL1 maintains inflorescence meristem (IM) and represses floral meristem (FM) formation. Since coordinate differentiation of three layers are essential for the floral primordia formation, TFL1 function is supposed to be required in the whole region of IM, but its transcript was localized only inner region of L3 layer of IM. We have revealed non-cell-autonomous function of TFL1 is conferred by its protein trafficking in the shoot apical meristem (SAM).

We have also found that FT moves among cell layers of IM. The endogenous FT is, however, expressed in the vascular bundle of leaf apical region, and recent study revealed that FT interacts with FD and functions in the SAM. Taken together, the following scenario can be deduced; after long distance travel from leaves via vascular bundle, FT is unloaded near the SAM (vascular bundle is undifferentiated in the SAM) and then FT protein moves to spread into the SAM and interacts with FD to activate target genes.

Another recent study suggests that FT RNA moves from the leaf to the SAM. To confirm this, we have developed grafting system using tobacco. As expected, transgenic tobacco carrying Arabidopsis FT gene showed early flowering, we used it as a rootstock and wild type scion was grafted. We found the scions acquired early flowering phenotype. Now we are investigating FT RNA and/or protein are transmitted from rootstock to scion by using modified FT genes.

148 Deletion of core components of the plastid protein import machinery causes differential arrest of embryo development in Arabidopsis thaliana

Bianca Hust, Michael Gutensohn
Institute of Plant Physiology, Martin-Luther-University Halle-Wittenberg, Germany

Among the genes that have recently been pinpointed to be essential for plant embryo development a large number encodes plastid proteins suggesting that embryogenesis is linked to plastid localized processes. However, nuclear encoded plastid proteins are synthesized as precursors in the cytosol and subsequently have to be transported across the plastid envelopes by a complex import machinery. We supposed that deletion of components of this machinery should allow a more general assessment of the role of plastids in embryogenesis since it will not only affect single proteins but instead inhibit the accumulation of most plastid proteins. Therefore we have characterized three Arabidopsis thaliana mutants lacking core components of the Toc complex, the protein translocase in the outer plastid envelope membrane, which indeed show embryo lethal phenotypes. Remarkably, embryo development in the atToc75-III mutant, lacking the pore forming component of the translocase, was arrested extremely early at the two-cell stage. In contrast, despite the complete or almost complete lack of the import receptors Toc34 and Toc159, embryo development in the atToc33/34 and atToc132/159 mutants proceeded slowly and was arrested later at the transition to the globular and the heart stage, respectively. These data demonstrate a strict dependence of cell division and embryo development on functional plastids as well as specific functions of plastids at different stages of embryogenesis. In addition, our analyses suggest that not all components of the translocase are equally essential for plastid protein import in vivo.

149 Mechanisms of Interaction and Consequences of Protein Complexes Involving AGAMOUS-Like 15 (AGL15), a MADS-Domain Transcription Factor that Preferentially Accumulates in the Plant Embryo

Kristine Hill[1], Huai Wang[2], Sharyn Perry[1]

[1]University of Kentucky , [2]University of Wisconsin

AGAMOUS-Like 15 (AGL15) encodes a MADS-box transcription factor that is preferentially expressed in the plant embryo, and is believed to be an important regulator in embryonic developmental programs. Research in our lab has identified a number of downstream targets of AGL15, and while some of these target genes are induced in response to AGL15, others are repressed. A number of direct target genes have been analyzed that exhibit strong responses to AGL15 levels in vivo, yet in vitro, AGL15 binds only weakly. Taken together these data suggested that AGL15 may form hetero-dimers, or ternary complexes with other proteins, thus modulating AGL15's specificity and function in planta. The Yeast Two-Hybrid system has been used to address this question and putative interactors of AGL15 have been identified. The mechanisms of interactions and the consequences of these associations in planta will be presented.

A motif within the C-terminal domain of AGL15, which is conserved in putative orthologs of AGL15, appears to function as a repression domain in vivo. One protein, identified in the Yeast Ttwo-Hybrid screen as a potential interacting protein of AGL15, is a member of the SIN3 histone deacetylase complex (HDAC), and preliminary data suggests that the aforementioned motif might mediate AGL15's association with, and recruitment of, members of this complex.

Co-Immunoprecipitation and Chromatin Immunoprecipitation (ChIP) approaches are being employed to study the biological functionality of protein-protein interactions involving AGL15 in planta, and to ultimately elucidate the subset of downstream targets regulated by AGL15 in conjunction with the different protein partners identified.

150 Characterisation of *fn10*, A Novel Germination Mutant In *Arabidopsis thaliana*

Tara Holman[1, 2], Steven Footitt[2], Freddie Theodoulou[2], Michael Holdsworth[1]

[1]University of Nottingham, Sutton Bonington Campus, Loughborough, Leics. LE12 5RD, UK, [2]Rothamsted Research, Harpenden, Herts. AL5 2JQ, UK

The regulation of germination is controlled by many genetic loci. A large number of these loci are involved in the biosynthesis and signalling of two phytohormones - abscisic acid (ABA) and gibberellins (GA). These two hormones have an antagonistic relationship: ABA promotes dormancy, GA promotes germination.

Seed dormancy is an evolutionary strategy that delays the germination of a proportion of the seed set until successive seasons. It also prevents the seed from germinating whilst attached to the mother plant.

Here we describe the positional cloning and the phenotypic characterisation of *fn10*, a novel germination mutant in Arabidopsis thaliana. This mutant was identified from a forward genetic screen for mutants with a reduction in germination potential. Also identified in this screen was the *cts-1* (*comatose-1*) mutant (Russell *et al.*, 2000; Footitt *et al.*, 2002).

Studies of the germination characteristics of *fn10* have shown that it requires a prolonged period to after-ripen, it is hypersensitive to ABA for germination, and seedlings fail to establish on sucrose. The germination potential of *fn10* can be increased with either exogenous GA or a period of stratification.

Double mutants have been constructed between *fn10* and *abi1-1* (*ABA insensitive-1*), *aba1-1* (*ABA deficient-1*), and *rgl2* (*RGA-like-1*) (Koornneef *et al.*, 1982; Koornneef *et al.*, 1984; Lee *et al.*, 2002). The *abi1-1* mutant is insensitive to ABA, the *aba1-1* is disrupted in ABA biosynthesis and the *rgl2* mutant displays constitutive GA signalling effects. Phenotypic analysis of the double mutants has shown that ABI1 is epistatic to FN10, and FN10 is epistatic to both ABA1 and RGL2.

A model for the action of FN10 in the regulation of germination is proposed.

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151 Dissection of Flowering Pathways Using a GL2-class HD-ZIP Protein FWA

Yoko Ikeda, Ayako Yamaguchi, Mitsutomo Abe, Takashi Araki

Department of Botany, Graduate School of Science, Kyoto University

Late-flowering mutant fwa is a dominant epigenetic mutant that ectopically expresses a GL2-type HD-ZIP gene due to promoter hypomethylation. Previous genetic analysis suggests that FWA blocks the flowering pathway at FT and/or downstream of FT. We envisage that FWA may provide a unique tool to dissect pathway from FT to flowering. Therefore, we investigated the mechanism by which ectopic FWA causes late flowering phenotype. First, we tested the possibility that FWA inhibits flowering through the transcriptional mis-regulation of target genes. We performed microarray analysis using two independent fwa epialleles and a 35S::FWA line. The transcriptional profiles of these plants suggest that late-flowering phenotype of fwa is unlikely due to the mis-regulation of transcription. Next, we examined interaction of FWA protein with known flowering regulators such as FT, TSF, TFL1 and FD. FWA protein strongly interacted with FT protein through its C-terminal region and ZIP domain in yeast cells. No interaction was observed between FWA and TSF, FWA and TFL1, and FWA and FD. Interaction between FWA and FT was confirmed by in-vitro pull down assay. C-terminal truncation of FWA abolished interaction with FT. Overexpression of FWA with truncated C-terminus did not cause late-flowering phenotype. These suggest that ectopically-expressed FWA inhibits floral transition by interfering with the FT function through protein-protein interaction. Based on this, we investigated the site of action of FT protein using FWA protein as a specific inhibitor of the FT protein function. We examined the tissue where FWA can exert its negative effect on flowering. FWA expressed in the shoot apex by FD promoter delayed flowering, while FWA expressed in the vascular tissues (including the phloem cells which express FT) did not. These results strongly suggest that FT protein acts in the shoot apex.

152 Arabidopsis SUMO-E3 ligase, AtSIZ1 Negatively Regulates Flowering Time Dependent of Autonomous and SA pathway

Jing Bo Jin[1], Yin Hua Jin[1], Ji Young Lee[2], Kenji Miura[1], Chan Yul Yoo[1], Dae-Jin Yun[2], Ilha Lee[3], Ray Bressan[1], Paul Hasegawa[1]

[1]Purdue.University, [2]Gyeongsang National University, [3]Seoul National University

Small ubiquitin-like modifier (SUMO) conjugation plays critical roles in many cellular processes. We have determined that AtSIZ1 is a negative regulator of flowering time through a salicylic acid (SA)-dependent and the autonomous pathways. *siz1-2* and *siz1-3* plants exhibit early flowering under short days , but only a modicum of earliness under long days.*siz1* does not alter circadian rhythm-dependent and *CO* or GI expression patterns relative to wild type and flowering of siz1 plants is accelerated to a similar extent as wild type in response to vernalization and GA treatments. Quantitative (q) PCR determination of transcript abundance and genetic analysis of double mutants (siz1-2 ft-1 and siz1-2 soc1-2) indicate that early flowering is attributable to *FT* and *SOC1*. siz1 plants have constitutively elevated SA levels and NahG reduces SA levels and partially suppresses early flowering of siz1-2 (i.e., siz1-2 NahG) plants. Early flowering of siz1 plants is linked to reduced FLC transcript abundance but FLM and MAF2 mRNA levels are similar to wild type. Flowering time evaluation of siz1-2, flc-3 and siz1-2 flc-3 plants flower earlier than single parental plant, indicates that early flowering phenotype of *siz1* is partially *FLC*-dependent and SA might be the second factor. Analysis of genetic interaction between several autonomous pathway genes (such as *LD*, *FCA* and *FVE*) and SIZ1, have shown that early flowering phenotype of *siz1* is suppressed by mutation in autonomous pathway genes *(ld-1, fca-9 and fve-3)*. These results indicate that down-regulation of *FLC* mRNA abundance in *siz1* is autonomous pathway dependent.

153 Novel insights into cell separation, abscission, apical dominance, epinasty and meristem arrest using dab4-1 (delayed abscission)

Joonyup Kim[1], Bradely Dotson[2], Camila Rey[2], Sara Patterson[1]

[1]University of Wisconsin-Madison, Cellular and Molecular Biology, Department of Horticulture, [2]University of Wisconsin-Madison, Department of Horticulture

Although remembered as one of the earliest traits selected by human being, it is not until recently that abscission has been recognized as a model for cell separation process. Abscission is the developmental process that includes a series of programmed events resulting in detachment of organs. We have been characterizing a novel floral organ abscission mutant, dab4-1 (delayed abscission) in Arabidopsis. This mutant has several unique phenotypes including delayed floral organ abscission, lack of anther dehiscence, delayed meristem arrest, epinastic leaf growth and strong apical dominance. Recent observations indicate that dab4-1 has altered responses to several phytohormones. To further understand the hormone interactions in dab4-1, expression of more than 20 plant hormone response genes were examined. Many of these genes were differently regulated in the mutant background. Levels of auxin in dab4-1 were measured both in the shoot apex and stem. In addition, the levels of methyl jasmonate (meJA) and jasmonic acid (JA) were measured for both in dab4-1 and wild type. Genetic interactions with other hormone response mutants have also been observed and will be discussed. We've cloned DAB4 using map-based cloning and it is an F-BOX gene on chromosome 2. Previous reports on this gene indicate that it is involved in the JA pathway and characterized for its mutants insensitivity to meJA, male sterility and mostly disease resistance. Here, we will demonstrate that dab4-1 has new additional phenotypes in specific ecotypes indicating novel roles of DAB4 during plant development. We are continuing further studies on protein-protein interactions along with downstream targets for DAB4 and believe this data will further elucidate novel functions of original DAB4 in plant development.

154 Functional Characterization of NEVERSHED and IDA, Genes Essential For Floral Organ Shedding in Arabidopsis

Michelle Leslie, Ji-Young Youn, Lalitree Darnielle, Michael Lewis, Emilee Glaub, Sarah Liljegren

University of North Carolina at Chapel Hill

Abscission is a specialized cell separation event that allows plants to shed mature organs, such as leaves, flowers and fruit. Recent work has shown that NEVERSHED (NEV) and INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) are each required for floral organ abscission in Arabidopsis. IDA is the founding member of a new class of putative signaling ligands (Butenko et al., 2003), and we have discovered that NEV is involved in vesicle trafficking. Currently, we are taking genetic and molecular approaches to further define the roles of NEV and IDA, explore their relationship, and elucidate the pathways that control cell separation. To identify additional components of the NEV pathway, we have carried out a suppressor screen for mutations that restore abscission in nev mutant flowers. Further characterization of these suppressors, including two allelic recessive suppressors that I am mapping, should identify proteins that interact with or downstream of NEV. In addition to our genetic characterization of NEV, we have developed NEV-specific antisera that we are using to test the co-localization of NEV with different endomembrane compartments. We are also testing whether the proper localization of IDA, which is predicted to be a secreted signal, may be dependent on NEV activity.

155 Dissecting genetic pathways underlying floral organ abscission

Michael Lewis, Michelle Leslie, Sarah Liljegren
UNC-Chapel Hill

We are using Arabidopsis as a model to study cell separation by focusing on the controlled shedding, or abscission, of floral organs after fertilization. Our lab has identified NEVERSHED (NEV) as a gene that is required for the abscission of sepals, petals and stamens. The cellular morphology of nev mutant flowers and activity of the NEV gene product suggest that it plays a role in vesicle trafficking. To identify factors that may physically interact with or downstream of NEV, we have conducted a screen for mutations that restore organ shedding in a nev background. Four recessive mutations have been isolated that confer nearly wild-type abscission in nev flowers as well as another recessive mutation that shows a partial rescue. We have also identified four dominant mutations that suppress the nev phenotype, including one which is intragenic. Characterization of the loci corresponding to these nev suppressors will facilitate further dissection of vesicle trafficking pathways required for plant cell separation.

156 *FOLDED PETALS* is involved in petal maturation in *Arabidopsis*

Noritaka Matsumoto[1], Seiji Takeda[2], Kiyotaka Okada[1]
[1]Department of Botany, Graduate School of Science, Kyoto University, [2]Department of Cell and Developmental Biology, John Innes Centre

Flower has four types of organs, sepals, petals, stamens, and carpels. Though well-known ABC model is explaining the mechanism of the decision of the floral organ identity, little is known about the maturation processes of floral organs. Morphologically, petals have relatively simple structure to other floral organs: matured petals consist of fewer layers of cells and fewer types of cells. This structural simplicity makes petals a good model system to analyze process of organ maturation in flower. The morphological aspects of petal maturation in *Arabidopsis* were described (Smyth et al., 1990), which contained three major phases. 1) Petal primordia appear between sepal and stamen primordia. 2) Petals elongate along the inside of sepals through narrow space between the sepal and anther. 3) Petals elongate rapidly to mature when flower opens. However, molecular and genetical mechanisms controlling these processes are still unclear.

To investigate the mechanism underlying the processes, we screened mutants with defects in petal morphology, especially in the process of the maturation. We isolated a mutant, *folded petals* (*fop*), which petals are folded when the flower opens. In *fop*, the position and the identity of floral organs were normal. Histological analysis showed that the petals started folding around when elongation began (the second phase shown above). The matured petals in *fop* showed no significant difference in size and shape from those in wild type, except for the folded form. *FOP* encodes a putative transmembrane protein with unknown function. To address the character of FOP protein, we are analyzing cellular localization of FOP:GFP fusion protein both in transgenic plants and in protoplasts of suspension cells. Analysis of *pFOP:GUS* transgenic plants revealed that *FOP* was expressed not only in petals but also in other floral organs. We will discuss about the relationship between the function of *FOP* and the petal maturation.

157 Reversing photoperiodic response by clock mutations in Arabidopsis

Tsuyoshi Mizoguchi[1], Sumire Fujiwara[1], Atsushi Oda[1], Kanae Niinuma[1], Takeomi Tajima[1], Riichiro Yoshida[1], Hiroshi Kamada[1], George Coupland[2]

[1]University of Tsukuba, Japan, [2]Max-Planck Institute for Plant Breeding, Germany

Fluctuations in the length of the day affect developmental processes and behaviors of many organisms. This phenomenon is called photoperiodism and allows detection of seasonal changes and anticipation of environmental conditions. In Arabidopsis, LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) each encode a myb protein essential for clock function and play important roles in photoperiodic flowering by controlling rhythmic expressions of GIGANTEA (GI), CONSTANS (CO) and FLOWERING LOCUS T (FT). Here we demonstrate a reversal of day-length response by lhy cca1 and propose novel roles of the oscillator components LHY and CCA1 in clock-dependent and -independent processes in Arabidopsis. We have identified short vegetative phase (svp) and early flowering 3 (elf3) as suppressor mutations of the late flowering phenotype of the lhy cca1 in continuous light condition. Functional interaction among LHY, CCA1, SVP and ELF3 will be discussed in more detail.

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158 DNA Binding Properties of TCP4, a Protein Involved in Leaf Morphogenesis in *Arabidopsis*

Pooja Aggarwal, Nirmalya Chatterjee, Utpal Nath

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012, India

The TCP family of DNA-binding transcription factors regulate diverse aspects of plant development including flower asymmetry, plant architecture and leaf morphogenesis. The molecular function of TCP protein is to suppress cell proliferation in the axillary meristem at very early stage of organ development. *CINCINNATA*, a *TCP* gene in *Antirrhinum majus*, controls leaf shape and surface curvature by regulating cell proliferation both spatially and temporally. The *Arabidopsis* orthologue of *CIN*, *TCP4*, also controls leaf morphogenesis the same way *CIN* does in *Antirrhinum*.

It has been hypothesized that *CIN* controls cell proliferation by regulating transcription of its downstream target genes. However, little is known about what these targets are, in case of CIN or TCP4. We have used Random Binding Site Selection (RBBS) assay to determine the consensus-binding site of TCP4/CIN, in order to identify their direct targets. We show that Both TCP4 & CIN bind to the same consensus site GTGGTCCC. By studying the binding properties of TCP4/CIN with mutated oligos, we show that the core sequence to which these proteins bind to is TGGNCC. The DNA-binding domain (TCP domain) of TCP4 is predicted to form a basic helix-loop-helix (bHLH) protein. We also demonstrate that the binding affinity of TCP4 to its target DNA is comparable to other bHLH proteins. Searching the promoter database of *Arabidopsis* with the TCP4 consensus site as target sequence has identified many putative direct targets of TCP4. Some of the direct targets have been validated *in planta* and their role in TCP4 function will be discussed.

159 HANABA TARANU (HAN) Organizes Axis and Root Formation in the Early Embryo

Tal Nawy[1], Chris Somerville[2], Wolfgang Lukowitz[1]

[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, [2]Carnegie Institution, Stanford, CA

Root formation in *Arabidopsis* is initiated early, coinciding with the elongation of the embryonic provascular cells and the definition of a visible apical-basal axis. In this process, the hypophysis, or uppermost cell of the otherwise extraembryonic filamentous suspensor, is recruited by the embryo and undergoes an asymmetric division that produces the founder cell of the root meristem. We have identified mutations in the gene encoding the GATA-type transcription factor HANABA TARANU (HAN) that have a dramatic and novel effect on axis and root formation in the early *Arabidopsis* embryo. In *han* mutants, the hypophysis does not divide and the basal tier of the embryo is populated by fewer, abnormally large cells. In contrast to other known mutants that lack basal pattern elements such as *monopteros*, *han* embryos often generate an adventitious root in the central region of the embryo later in development. Analysis of basal marker gene expression in the *han* background is consistent with this observation, as basal fates are not lost but appear to shift apically. Interestingly, the embryonic auxin maximum as visualized by DR5:GFP also shifts from the hypophysis to the embryo proper in *han* mutants. We are currently exploring the question of whether HAN is involved directly or indirectly with auxin signaling, or if it operates in an independent pathway to pattern the early embryo.

160 Morphological changes in Arabidopsis by clock mutations lhy cca1 and prr9 prr7 prr5 in light/dark cycles and continuous light condition

Kanae Niinuma[1], Sumire Fujiwara[1], Norihito Nakamichi[2], Takeshi Mizuno[2], Hiroshi Kamada[1], Tsuyoshi Mizoguchi[1]

[1]University of Tsukuba, Japan , [2]University of Nagoya, Japan

LHY and CCA1 are shown to be closely associated with circadian clock function in Arabidopsis (1). The lhy cca1 mutation causes an extremely early flowering under short-days (1-3). We found that lhy cca1 flowered later than wild-type plants under continuous light (LL). The lhy cca1 plants showed dark green and curled leaves and short hypocotyls in LL. Molecular mechanisms of the dramatic morphological changes in light/dark (L/D) cycles and LL have not been elucidated. PSEUDO RESPONSE REGULATORS (PRR1/TOC1, PRR3, PRR5, PRR7 and PRR9) are also shown to be closely associated with circadian clock function in Arabidopsis (4). Triple loss-of-function of prr9 prr7 prr5 has been shown to cause an arrhythmic expression of clock-controlled genes in LL (4). The prr9 prr7 prr5 showed a late flowering phenotype and had pale green leaves and long petiols and hypocotyls in L/D cycles such as long-days and short-days (4). Here we demonstrate that the prr9 prr7 prr5 plants had dark green/ curled leaves and short petiols in LL. These are completely opposite to those in L/D cycles. The reversal of leaf color and petiol length phenotypes between L/D cycles and LL is common in lhy cca1 and prr9 prr7 prr5. Molecular mechanisms underlying the reversal of the phenotypes will be discussed.

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2) Mizoguchi et al. Plant Cell 2005
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161 Global and Locus-Specific Roles for Arabidopsis Paf1C Homologs in Transcription and Chromatin Modifications

Sookyung Oh, Steven van Nocker

Program in Plant Breeding and Genetics, Michigan State University, East Lansing, Michigan 48824

RNA Polymerase II-Associated Factor 1 Complex (Paf1C) in budding yeast plays a key role in reinforcing transcriptional activity by mediating the establishment and/or maintenance of specific chromatin modifications, promoting elongation and linking Pol II with elements of pre-mRNA processing machinery. This transcription factor is associated with chromatin at all canonical transcriptional units yet investigated and therefore probably plays a general transcriptional role.

Although components of Paf1C are conserved in higher eukaryotes, their potential mechanism in transcription has not been explored. In Arabidopsis, Paf1C subunit homologs are encoded by the VERNALIZATION INDEPENDENCE (VIP) genes. We found that loss of VIP gene function affects a substantial portion of the transcriptome, but strongly silences only a small subset of genes including the FLC/MAF family of MADS-domain flowering regulators.

To better understand the mechanism of VIP proteins in transcription, we characterized genome-wide and locus-specific effects of loss of VIP proteins on histone modifications and Pol II distribution. We analyzed methylation and acetylation sites (Lys-4, Lys-9, Lys-14, Lys-36) on the major canonical and variant histone H3 proteins, and found that at least VIP3 does not play a significant role in establishing these modifications when evaluated on a whole-chromatin level. However, we found that VIP proteins are required for specific chromatin modifications within a subset of VIP-dependent genes. Loss of VIP3 resulted in a decrease of Pol II density throughout FLC chromatin, including the promoter regions, suggesting the major influence on FLC expression is through Pol II recruitment and transcriptional initiation rather than elongation or pre-mRNA processing.

162 Female sporophytic Arabidopsis mutants impaired in pollen tube guidance

Yiding Huang, Ravi Palanivelu

Univeristy of Arizona, Univeristy of Arizona

A pollen tube's journey to an egg cell within the pistil involves cell to cell interactions such as attraction, repulsion and adhesion. This journey begins with pollen tube growing between the walls of the stigma cells, travelling through the extracellular matrix of the transmitting tissue, and finally arriving at the ovary, where it migrates up the funiculus, and enter the micropyle of an ovule to deliver the two sperm cells, one fertilizes an egg and other the central cell. Thus a pollen tube navigates past several different female cells before it reaches the egg and seeds are not produced if the pollen tube guidance process is disrupted. To isolate pollen tube guidance signals from female sporophytic cells, we performed a mutant screen by visually selecting Arabidopsis plants with reduced seed containing siliques from among the SALK TDNA insertion collection. Mutations that cause reduction in seeds for other reasons besides pollen tube growth and guidance defects male sporophytic and gametophytic defects (reduced or lack of pollen), pollination defects (reduced or lack of pollen on the stigma), embryo and endosperm lethality (shriveled seeds) and fully penetrant female gametophytic defects (~ half the number of seeds) were eliminated from the screen. To date, a primary screen of 5000 lines resulted in twenty candidate mutants. These candidate lines were then subjected to a series of assays to select only those lines that are impaired in pollen tube guidance. Based on the progeny analyses and reciprocal crosses to wild type plants we confirmed that at least two of these lines are impaired in pollen tube guidance due to female sporophytic tissue specific mutations. Progress on microscopy and in vitro pollen tube guidance analyses of mutants, and cloning the genes that are tagged with TDNA will be reported.

163 What are SCAMPS doing in plants? characterization of dab5-1, a delayed abscission mutant in Arabidopsis

Hongyu Rao[1], Karianne Kusner[1], Melinka Butenko[2], Wuyi Wang[3], Joshua Lindsey[4], Sara Patterson[1]

[1]Department of Horticulture, Cellular and Molecular Biology Program, University of Wisconsin -Madison, Madison, WI 53706, USA, [2]Department Molecular Biology, University of Oslo, Oslo Norway, [3]Ceres, Thousand Oaks, CA , [4]Department of Surgery, Medical School, University of Wisconsin-Madison, Madison, WI 53706, USA

The study of leaf abscission in plants was pioneered in the early 1900s by Neljubov with the observation that ethylene gas from streetlamps caused early senescence and abscission. Subsequent studies focused on the anatomy of the abscission zone of tomato Lycopersicum, tobacco and Sambucus, and transmission electron micrograph observations on these abscission zones indicated that during abscission increased vesicle trafficking and Golgi activity occurred. In following years, these studies were complemented by biochemical studies and a significant amount was ascertained about some of the enzymes involved in cell wall degradation. Although much of the focus on abscission has focused on cell wall associated genes, many researchers have speculated that changes in signaling in the secretory pathway may also directly regulate the abscission process. This hypothesis is not really new, as we know that abscission is an active process associated with increased vesicle trafficking; and consequently, it is logical to predict that mutations in genes regulating the secretory pathway would disrupt the abscission process. Despite these predictions, many of the genes in Arabidopsis that have been identified to have a role in vesicle trafficking have no phenotypic changes when disrupted. Using a forward genetic screen for mutants with delayed floral organ abscission, we have identified a T-DNA insertion upstream of a secretory membrane carrier protein (SCAMP) in dab5-1 that is responsible for delayed abscission. SCAMPs have been studied in mammalian systems and are associated with endocytic and exocytic trafficking. We will present characterization of the dab5-1 mutant, isolation and cloning of the T-DNA insertion, molecular complementation, and gene expression.

164 Constructing a Gene Regulatory Network for the Arabidopsis Female Gametophyte

Jayson Punwani, David Rabiger, Gary Drews

Department of Biology, University of Utah, Salt Lake City, UT 84112

The female gametophyte (FG) plays an essential role in plant reproduction. The *Arabidopsis* FG is a seven-celled structure composed of one central cell, one egg cell, two synergid cells, and three antipodal cells. How these cells acquire their unique features and functions during development is not understood. As an entryway to dissecting the gene regulatory networks directing cell specification and differentiation during FG development, we identified a group of genes expressed in the *Arabidopsis* FG. One of the FG-expressed genes we identified, *MYB98*, is a member of the R2R3-MYB family of transcription factors. The *MYB98* gene is expressed in the synergid cells of the FG. *myb98* FGs have defects in pollen tube guidance and formation of the filiform apparatus [Kasahara et. al., (2005) Plant Cell 17:2981-2992]. These data suggest that MYB98 functions as a transcription factor within the synergid cell gene regulatory network and controls the expression of a battery of downstream genes required for pollen tube guidance and formation of the filiform apparatus. To identify genes downstream of *MYB98*, we used real-time RT-PCR to screen for genes that are downregulated in *myb98* FGs. We have identified more than 10 genes via this screen. Using promoter:reporter constructs, we have shown that these genes are expressed in the synergid cells of wild-type FGs, but not *myb98* FGs. To determine the *cis*-regulatory elements required for expression within the synergid cells, we are dissecting the promoters of these genes. To determine the MYB98 consensus DNA binding site, we are using the selected and amplified binding site (SAAB) assay. To ascertain the function of these downstream genes, we are analyzing the phenotypes of mutants and determining the subcellular localization of GFP fusion proteins.

165 The Arabidopsis TALE Homeobox Gene *ATH1* Controls Flowering Time by Regulating *FLC* Levels

Bas Rutjens, Marco Brand, Sjef Smeekens, Marcel Proveniers

Molecular Plant Physiology, Utrecht University, Padualaan 8 3584 CH Utrecht, The Netherlands

Floral induction is controlled by a large number of genes acting in different pathways that either repress or promote the reproductive transition at the shoot apical meristem. Here we show that altered expression of the light-regulated *ARABIDOPSIS THALIANA HOMEOBOX 1* gene (*ATH1*), a member of the BEL family, results in a flowering time phenotype. We found that *ATH1* specifically affects mRNA levels of *FLOWERING LOCUS C* (*FLC*), the most prominent floral repressor in *Arabidopsis*. Basal levels of *FLC* are attenuated by *ath1*mutations resulting in early flowering. Interestingly, constitutive ectopic expression (OE) of *ATH1* does not increase *FLC* levels unconditionally. We noticed that weak *FLC* alleles are most effectively upregulated by *ATH1-OE* as is the case in backgrounds that carry the strong *FLC* activator *FRIGIDA* (*FRI*). Likewise, introduction of an *ath1* mutation in plants containing dominant *FRI* and *FLC* alleles partially suppresses the ability of *FRI* to increase *FLC* levels. Currently we are pinpointing the position of *ATH1* in the web of flowering time genes.

166 Two Novel Cycling Dof (DNA binding with one finger) Transcription Factors are involved in Flowering Time Regulation

Nicolas Sauerbrunn[1], Takato Imaizumi[2], Steve A. Kay[2], Richard Amasino[3]

[1]Max Planck Institute for Plant Breeding, Carl-von-Linne Weg 10, D-50829 Cologne, Germany, [2]Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA , [3]Department of Biochemistry University of Wisconsin Madison, WI 53706-1544

We identified two novel DOF-domain proteins (*CDF5 and CDF6*) which are closely related to *CDF1* (*Cycling Dof-Factor 1*). A constitutive over-expression of *CDF5 and CDF6* using the *CaMV 35S*-promoter leads to a late flowering phenotype whereas T-DNA insertion alleles of *CDF5* showed an early flowering phenotype in long day conditions.

The mRNA-levels of *CDF5 and CDF6* remain rhythmic in constant light conditions indicating that both genes are controlled by the circadian clock. Transgenic lines expressing *CDF5 and CDF6*-promoter:*GUS* constructs showed a GUS-expression in the root and leaf vascular tissues and in the stomata which was also the site of expression for *CDF1*. The CDF1-protein was shown to bind the *CONSTANS (CO*)-promoter and to act as a repressor of *CO*. The zinc-finger protein CO is a key regulator for the photoperiodic flowering pathway. In a Yeast two Hybrid assay *CDF5 and CDF6* interacted with the FKF1-kelch domain (FLAVIN-BINDING, KELCH REPEAT F-BOX 1) indicating that *CDF5 and CDF6* might be regulated by FKF1 at the protein level as has been shown for CDF1. FKF1 is thought to be a component of a Skp1-Cullin-F-box (SCF) E3 ubiquitin ligase complex and was shown to be involved in the degradation of CDF1.

Our current data suggests that *CDF5 and CDF6* are novel members of a transcription factor family which is redundantly involved in the flowering time control in *Arabidopsis thaliana*.

167 Analysis of DNA methylation and expression of embryogenesis-related genes in plants

Tomiko Shibukawa, Akira Kikuchi, Hiroshi Kamada

University of Tsukuba, Japan

DNA methylation is known to involve in the regulation of gene expression. In animals, the hypomethylation of DNA leads to the aberrant embryogenesis with abnormal expression of embryogenesis-related genes. On the other hand, in plants, there is limited information about the relation between DNA methylation and embryogenesis.

In this study, we investigated the relation between DNA methylation and expression of embryogenesis-related genes in plants (e.g. LEC1, ABI3 and FUS3 in Arabidopsis, C-LEC1 and C-ABI3 in carrot). The expression of embryogenesis-related genes was examined in various tissues (cotyledon, rosette leaf, root, apical tip, flower bud, flower and somatic embryo) of Arabidopsis mutants deficient in DNA-methylation-related genes (e.g. ddm1 and cmt3). Some genes showed different expression profiles in the mutants as compared to the wild type. In carrot, there are some induction systems of somatic embryogenesis, which provide a large amount of synchronously developing embryos. Using the system, we tried to clarify the relationship between the gene expression of embryogenesis-related genes (C-LEC1 and C-ABI3) and DNA methylation on the genomic region during embryogenesis by Sourthen blot analysis, using isoschizomeric restriction enzymes and bisulfite sequencing procedure. These results suggest that DNA methylation may involve in the regulation of expression of embryogenesis-related genes.

168 Genetic Dissection of Parental Effects in Seed Development

Reza Shirzadi[1], Moritz Nowack[2], Reidunn Aalen[1], Arp Schnittger[2], Paul Grini[1]

[1]Department of Molecular Biosciences, University of Oslo, Norway, [2]2University Group at the Max-Planck-Institute for Plant Breeding Research, Cologne, Germany

Seed development requires a coordinated interplay of the embryo, the endosperm and the maternal seed coat. The embryo and the endosperm are the products of the double fertilization of the egg cell and the central cell by two sperm cells from the pollen. What roles gametophytic parental (maternal and paternal) transcriptional programmes play in this process is not clear. We have used the recently described cdc2a cell cycle mutant as a tool to dissect the involvement of maternal and paternal gene programs in seed development. In the paternal effect cdc2a mutant line, mutant pollen fail to undergo the second pollen mitosis, resulting in pollen with only one sperm cell instead of two. The single sperm cell from cdc2a mutant pollen is able to successfully and exclusively fertilize the egg cell. cdc2a fertilized seeds eventually arrest and abort, but although not fertilized, the central cell breaks the mitotic block and starts developing (autonomous) endosperm. This identifies a novel positive signal from the fertilization of the egg cell that triggers endosperm development. We have analyzed transcriptional profiles of cdc2a induced seed development and have identified several genes that that show significant up- or down regulation compared to wild-type fertilization. The polycomb FIS-group of genes also produce autonomous endosperm development when mutated, and are repressors of endosperm proliferation in the absence of fertilization. One way of explaining seed abortion in cdc2a fertilization products is thus the repression of endosperm development by active FIS-group genes. To test this hypothesis we fertilized FIS-group mutants with cdc2a pollen. In the progeny of the crosses a up to three-fold higher frequency of cdc2a mutant plants could be found, showing that cdc2a triggered diploid endosperm could support vital seed development in the absence of repression by the FIS-group genes. This allows seed development without any paternal contribution to the endosperm and opens for an extensive exploration of the transcriptional contribution of the paternal genome to endosperm development.

169 Identification of Genes Expressed in the Arabidopsis Female Gametophyte

Joshua Steffen, Il-Ho Kang, Jane Macfarlane, Gary Drews
University of Utah

The *Arabidopsis* female gametophyte (FG) is composed of 1 egg cell, 1 central cell, 2 synergid cells, and 3 antipodal cells. The FG plays a role in many stages of the reproductive process including pollen tube guidance, double fertilization, induction of seed development, and maternal control of embryo and endosperm development. The molecular processes by which the female gametophyte cells acquire their unique features and functions during cell differentiation are not understood. As a first step toward dissecting the gene regulatory networks controlling cell specification and differentiation during FG development, we have used expression assays to identify a collection of genes expressed in the *Arabidopsis* FG.

We carried out a differential microarray screen to identify genes expressed in the female gametophyte. Our general strategy was to identify mRNAs present in WT ovules but not in mutant ovules lacking FGs. Using microarrays we identified 86 genes that have reduced expression in mutant ovules. To validate the expression patterns of these genes, we carried out real-time RT-PCR assays with RNA from independently harvested ovules. Using the criteria of ≥8-fold change, 71 of the 86 genes were validated as having reduced expression in mutant ovules. To determine which cells within the female gametophyte these genes are expressed within, we generated *Promoter:GFP* gene fusions. Analysis of promoter-GFP transgenic plant is in progress. The identification of genes expressed within specific cells of the FG allows us to take the first steps towards dissecting gene regulatory networks controlling cell differentiation during FG development.

170 Direct regulation of the floral homeotic APETALA1 gene by APETALA3 and PISTILLATA in Arabidopsis

Jens Sundstrom[1, 2], Naomi Nakayama[2], Kristina Glimelius[1], Vivian Irish[2]
[1]Swedish Agricultural University, [2]Yale University

The floral homeotic gene APETALA1 (AP1) specifies floral meristem identity and sepal and petal identity in Arabidopsis. Consistent with its multiple roles during floral development, AP1 is initially expressed throughout the floral meristem, and later its expression becomes restricted to sepal and petal primordia. Using Chromatin Immunoprecipitation we show that the floral homeotic PISTILLATA (PI) protein, required for petal and stamen development, has the ability to bind directly to the promoter region of AP1. In support of the hypothesis that PI, and its interacting partner APETALA3 (AP3), regulates the transcription of AP1 we show that AP1 transcript levels are elevated in strong ap3-3 mutant plants. Kinetic studies, using transgenic Arabidopsis plants in which both AP3 and PI are under posttranslational control, show that AP1 transcript levels are down regulated within 2 hours of AP3/PI activation. This implies that the reduction of AP1 transcripts is an early event in the cascade following AP3/PI induction and provides independent support for the hypothesis that AP1 is a direct target of the AP3/PI heterodimer. Together, these results suggest a model whereby AP3/PI directly acts, in combination with other factors, to restrict the expression of AP1 during early stages of floral development.

171 Identification of TIA as a Myb-like Protein Affecting Multiple Aspects of Development

Ludmila Tyler, Tai-ping Sun

DCMB Group, Department of Biology, Duke University, Durham, NC U.S.A.

Plant development requires the coordination of numerous inputs, including hormonal signals. Bioactive gibberellins (GAs) are phytohormones that regulate developmental processes ranging from seed germination to vegetative growth, the transition to flowering, and floral organ formation. Through an activation-tagging screen to identify novel components of the GA signaling pathway, a tall, late-flowering transformant with increased apical dominance was identified in the partially GA-deficient ga1-6 background. Activation tagging involves introducing a series of enhancers randomly into the genome to cause the over-expression of genes adjacent to the insertion sites. The identified line over-expressed a gene for a putative Myb-like transcription factor of the GARP family. The tagged gene has been named *TIA* for *TALL, INCREASED APICAL DOMINANCE*. Although the increased height and apical dominance of the activation-tagged line could be consistent with increased GA response, the delayed flowering is opposite to what one would expect for an up-regulation of GA signaling. While TIA may be only indirectly connected to the GA pathway, this gene does appear to modulate plant development. Over-expression of *TIA* in a wild-type background not only recapitulated the tall, late-flowering phenotype, but also generated plants with thick primary stems, aerial rosettes, and occasional flowers with abnormal petal numbers. A fusion of TIA with green fluorescent protein exhibited nuclear localization, as would be expected for a transcription factor. Also, quantitative-PCR-based analysis and the characterization of promoter-reporter constructs indicated that TIA is endogenously expressed in the plant vasculature throughout development. In addition, several independent TIA RNA interference (RNAi) lines flowered early. Together with the RNAi phenotype, the pleiotropic effects of over-expressing TIA suggest that this novel, putative transcription factor regulates multiple aspects of development, including flowering-time.

172 Functional analysis of AtVps9a, the sole activator of Rab5-related GTPases

Wakana Uchida[1], Tatsuaki Goh[1, 2], Satoko Arakawa-Kobayashi[1], Masaki Takeuchi[1, 3], Ken Sato[1], Takashi Ueda[2], Akihiko Nakano[1, 2]

[1]RIKEN Discovery Research Institute, Wako, Saitama, Japan, [2]Dept. of Biological Sciences, Graduate School of Science, University of Tokyo, Hongo, Tokyo, Japan, [3]Present Address: Dept. of Molecular Structure, Institute for Molecular Science, National Institutes of Natural Sciences, Nagoya, Japan

Plant endocytosis plays important roles in polar transport of auxin, establishment of cell polarity, cell plate formation during cytokinesis, cell wall morphogenesis and so on. To understand molecular mechanisms of plant endocytosis, we have been focusing on Rab5 GTPase-mediated endocytosis. In animal cells, Rab5 is known to organize many events in early endocytic pathway, such as homotypic fusion between early endosomes, alteration of lipid composition of the endosomal membrane, and signal transduction through endosomes via specific interactions with effector proteins. Rab5 is activated by the guanine nucleotide exchange factor(s) (GEF). In animals, different classes of Rab5 GEFs regulate Rab5 activity in distinct steps of the endocytic pathway. In *Arabidopsis thaliana*, there are three Rab5-related GTPases, Ara7, Rha1 and Ara6. As for the structure, Ara7 and Rha1 are similar to animal-type Rab5 while Ara6 is unique to plants. We found that only one Rab5 GEF, AtVps9a, can activate all the Rab5 members in Arabidopsis. In the *atvps9a-1* mutant whose GEF activity is completely lost, embryogenesis is arrested at the torpedo stage. In the *atvps9a-2*, a leaky allele lacking the C-terminal regulatory domain, elongation of the primary root was severely affected. The *atvps9a-1* embryo and the *atvps9a-2* primary root exhibited similar abnormal morphologies at the cellular level; 1) cells hypertrophied and aligned irregularly and 2) cell plate formation and cell wall morphology were aberrant. Electron microscopy demonstrated the accumulation of vesicles derived from Golgi, endosome-like structures and aberrant membranes in the *atvps9a-1* embryo cells. A genomic fragment containing the *AtVPS9a* region restored all phenotypes observed in these mutants. These results indicate that AtVps9a plays essential roles in the plant development.

173 Flowering Time Control in Arabis alpina, a Perennial Relative of Arabidopsis thaliana

Renhou Wang[1], Maria Albani[1], Coral Vincent[1], Franziska Turk[1], Fabio Fornara[1], Carlos Blanco[2], George Coupland[1]
[1]Department of Plant Developmental Biology, Max-Planck-Institute for Plant Breeding Research, Koln, Germany, [2]Departamento de Genetica Molecular de Plantas, Centro Nacional de Biotecnologia, CSIC, Madrid, Spain

To study flowering-time control in polycarpic perennials and perennialism-related traits such as juvenility, we have developed Arabis alpina as a model species. As a member of the Brassicacea family, A.alpina is closely related to Arabidopsis thaliana, which makes it easy to transfer to Arabis alpina the knowledge on flowering acquired in Arabidopsis. Arabis alpina has other important attributes that make it an attractive model species such as being diploid and able to self fertilize.

We have investigated juvenility in Arabis alpina, a trait not shown by Arabidopsis. Arabis alpina has a four-week-long juvenile phase, during which the plant does not flower in response to floral inductive signals, such as vernalization treatment, that are sufficient to cause adult plants to flower. To unravel the underlying mechanisms, homologues of Arabidopsis flowering time and floral meristem identity genes, FLC, TFL1, FT, SOC1, and LFY have been isolated from A.alpina and named AaFLC, AaTFL1, AaFT, AaSOC1, and AaLFY respectively. The expression patterns of these genes were tested in juvenile and adult plants. The results suggest high levels of AaTFL1 in the meristems of juvenile plants might correlate with juvenility.

Another interesting issue we are studying in A.alpina is the genetic basis of natural variation in flowering time of different accessions. Two accessions with distinct vernalization requirements for flowering have been identified. The Pajares accession has an obligate requirement for vernalization to promote the floral transition whereas the Bonn accession flowers early without vernalization. Analysis of expression of different flowering-time genes showed that AaFLC was expressed at similarly high levels in both accessions while homologues of the floral pathway integrator genes, AaFT and AaSOC1 were expressed at higher levels in the Bonn accession than in the Pajares one. Genetic analysis indicated that the distinct vernalization requirements for flowering in these two Arabis alpina accessions mainly arise from a locus other than AaFLC and the allelic variation at this locus does not affect AaFLC expression levels. This observation is in contrast to Arabidopsis where most of the natural variation in vernalization is either at FLC or FRI, which regulates FLC expression.

174 A novel regulatory pathway that promotes flowering in response to UV light identified by high-throughput misexpression of Arabidopsis transcription factors

Stephan Wenkel, Lionel Gissot, Jose Le Gourrierec, Aneta Domen, George Coupland
Max-Planck-Institute for Plant Breeding Research

In order to isolate novel transcription factors that are involved in controlling flowering in Arabidopsis, we performed a large-scale misexpression screen. Around 1,000 Arabidopsis transcription factors were expressed under the *SUC2*-promoter in the phloem companion cells, where *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) are expressed and act to promote flowering. Screening of T1-transformants revealed several late and early flowering lines misexpressing transcription factors that belong to different families. We misexpressed 69 *APETALA2-LIKE* transcription factors and of these 62 had no effect on flowering time, four caused late flowering and three caused early flowering. In a parallel approach we identified proteins that bind to the *FT* promoter by yeast-one-hybrid screening. One of the interacting proteins was named FIDGET (FIT). *FIT* caused early flowering when expressed under the *SUC2* promoter. The early flowering phenotype of *SUC2::FIT* is caused by upregulation of *FT*. Furthermore, we demonstrate that FIT binds to the *FT* promoter in yeast, *in vitro* and *in vivo*. We tested to which environmental cues *FIT* expression responds and found that it is highly induced upon UV treatment. UV light also induces *FT* expression and accelerates flowering in an *FT* dependent manner. Two other AP2-like transcription factors that caused late flowering can also interact with the *FT* promoter in yeast. This suggests that UV as well as other stress-related pathways, involving AP2-like transcription factors, converge on the control of *FT* expression to regulate flowering time in response to environmental stimuli.

175 DEMETER DNA Glycosylase Regulates MEDEA Polycomb Gene Self-Imprinting and Seed Viability by Allele-Specific Demethylation

Wenyan Xiao, Mary Gehring, Jin Hoe Huh, Tzung-Fu Hsieh, Jon Penterman, Yeonhee Choi#, Robert Fischer*
Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

We isolated mutations in Arabidopsis to understand how the female gametophyte controls embryo and endosperm development. Maternal mutant dme or mea alleles result in seed abortion. DEMETER (DME) encodes a large protein with DNA glycosylase and nuclear localization domains. DNA glycosylases initiate the base-excision DNA repair pathway by excising damaged or mispaired bases. An invariant aspartic acid in the active site is involved in catalyzing the excision reaction. We mutated the invariant aspartic acid at position 1304 in DME to asparagine and found that the conserved aspartic acid residue is necessary for DME to function in vivo and in vitro. DME is expressed primarily in the central cell of the female gametophyte, the progenitor of the endosperm. MEDEA (MEA) is a Polycomb group gene that is imprinted in the endosperm. The maternal allele is expressed and the paternal allele is silent. DME DNA glycosylase activates maternal MEA allele expression in the central cell. We identified mutations that suppress dme seed abortion and found that they reside in the METHYLTRANSFERASE1 (MET1) gene, which maintains cytosine methylation. DME and MET1 are antagonists in the central cell. DME activates whereas MET1 suppresses maternal MEA:GFP allele expression. MET1 methylates the maternal MEA allele whereas DME causes maternal-allele-specific hypomethylation at the MEA gene. DME excises 5-methylcytosine in vitro and in E. coli. We propose that excision of 5-methylcytosine by DME, followed by insertion of cytosine by downstream enzymes in the base excision DNA repair pathway, results in DNA hypomethylation and expression of the maternal MEA allele. Unexpectedly, paternal-allele silencing is not controlled by DNA methylation. Rather, Polycomb group proteins that are expressed from the maternal genome, including MEA, are required for paternal MEA silencing. Thus, DME establishes MEA imprinting by removing 5-methylcytosine to activate the maternal allele. MEA imprinting is subsequently maintained in the endosperm by maternal MEA silencing the paternal MEA allele. New approaches undertaken to further understand mechanisms for DME-regulated gene imprinting and seed development will be discussed.

Present Address:
*Fred Hutchinson Cancer Research Center, Seattle, WA 98109
#Dept of Biological Sciences, Seoul National University, South Korea

176 VAJURA, an EF-2 Family Protein, Is Involved in Flower Development and Gametophytogenesis

Noriyoshi Yagi[1], Seiji Takeda[2], Noritaka Matsumoto[1], Kiyotaka Okada[1]
[1]Department of Botany, Graduate School of Science, Kyoto University, [2]Department of Cell and Developmental Biology, John Innes Centre

In eukaryotes, pre-mRNA splicing is an essential process for gene expression. The splicing occurs in a large complex, the spliceosome, which consist of five small nuclear ribonucleoprotein particles (snRNPs; U1, U2, U4/U6, and U5) and other non-snRNP splicing factors. The splicing machinery has been studied extensively using mammalian and yeast cells. In *Arabidopsis*, one of multicellular organisms, recent studies identified many splicing factors, but little is known about the role of pre-mRNA splicing in plant development.

The *VAJURA* (*VAJ*) gene encodes an EF-2 family protein, of which counterparts in human and yeast are one of the components of U5 snRNP. It is known that, in plants, spliceosomal components localize in Cajal bodies and in nuclear speckles. Transient expression assay using *Arabidopsis* protoplast revealed that VAJ:mRFP co-localized with SC35:GFP, speckle marker. Sequence similarity and subcellular localization suggest that VAJ functions as a splicing factor.

To assess the role of *VAJ* during developmental process, we characterized *vaj* mutants. In *vaj-1*, a weak allele, sepals and petals were narrower and longer than those of wild type. Although homozygous plants of *vaj-1* were not lethal, in *vaj-2* and *vaj-3*, T-DNA insertion lines, no homozygote was identified from self-pollinated heterozygous plants. The segregation ratio of heterozygote and wild type indicated that *vaj-2* and *vaj-3* mutations affected gametophyte development. We are now performing reciprocal crossing test and cytological studies of male and female gametophyte.

A *VAJ*-related gene, *VAJURA-LIKE* (*VAL*) was found in *Arabidopsis*. The *val* mutant showed no obvious phenotype. EST of *VAL* was not found at database suggesting that *VAL* is not expressed or is expressed at very low level. To examine *VAL* function genetically, we are generating *VAJ*/*vaj val*/*val* mutant. What kind of role *VAJ* and *VAL* play in plant development will be discussed.

177 Signaling of Anther Cell Fate Determination by the EMS1 Protein Kinase in *Arabidopsis*

Dazhong Zhao, Gengxiang Jia

Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA

In flowering plants, male gametophytes develop in anther where cell differentiation and subsequent degeneration are essential for successful reproduction. The anther contains highly specialized cell types. The reproductive cells, microsporocytes (pollen mother cells), undergo meiosis and eventually develop into pollen grains. The remaining non-reproductive cells (somatic cells), including epidermis, endothecium, middle layer and tapetum, are required for the normal development and release of pollen. However, very little is known about the molecular mechanisms of cell fate determination during anther development. The *Arabidopsis* mutant, *excess microsporocytes1* (*ems1*), produces excess microsporocytes and lacks tapetal cells. The fact that the number of excess microsporocytes in the mutant is close to the sum of wild-type microsporocytes and tapetal cells suggests that the tapetum precursor cells differentiate into microsporocytes in the mutant. The *EMS1* gene encodes a leucine-rich repeat receptor-like protein kinase (LRR-RLK), and its expression is associated with the differentiation of the microsporocytes and tapetal cells, indicating that EMS1 mediates signals that control cell fate determination during anther development.

178 Mapping binding sites for the MADS-factor AGL15: a custom ChIP-chip approach

Yumei Zheng[1], Weining Tang[2], Sharyn Perry[1]

[1]University of Kentucky, [2]Emory University School of Medicine

AGL15 is a MADS-domain containing regulatory factor that accumulates primarily, although not exclusively, during embryogenesis. Overexpression of this gene promotes somatic embryo development in several systems. To better understand AGL15's function, both in plant development and in promoting somatic embryogenesis, it is necessary to identify and characterize genes whose expression is controlled by AGL15. We have used a chromatin immunoprecipitation (ChIP) approach to identify DNA fragments directly bound by this protein. This has been successful and demonstrated that at least one mechanism by which AGL15 promotes somatic embryo development from the shoot apex of seedlings in a liquid culture system, is by controlling biologically active GA amounts (Wang et al., 2004, Plant Cell 16, 1206-1219). However, ChIP is a low-throughput method. Therefore, we are currently utilizing a custom ChIP-chip approach wherein putative targets of AGL15 are spotted on a glass slide and hybridized with fluorescently labeled probe. This relatively inexpensive method allows rapid discrimination of true binding sites from background and allows some measurement of the potential occupancy of the site. Paired with expression microarrays, it will be possible to determine consequences of binding, as well as identification of genes directly regulated by AGL15 from those that may be indirectly regulated. Additionally it is possible to hybridize these chips with probe derived by ChIP from a variety of developmental stages and tissues, allowing insights into overlap of developmental programs. Finally this resource will be useful to identify which DNA fragments that AGL15 and particular interacting proteins both bind. Experience with this ChIP-chip will be presented.

179 Investigation Of The Connection Between Leaf Polarity And Meristem Formation Through Enhancers Of pinhead

Debbie Alexander, Karyn Lynn Newman, M. Kathryn Barton
Carnegie Institution of Washington

Several lines of evidence indicate that the adaxial leaf domain possesses a unique competence to promote shoot apical meristem formation and maintenance. The Arabidopsis PINHEAD (PNH) gene likely plays a role in this process as PNH is expressed in adaxial domains and loss of function pnh mutants fail to maintain the indeterminacy of shoot meristems. Furthermore, PNH acts redundantly with ARGONAUTE1 (AGO1) to control organ polarity. PNH and AGO1 are closely related members of the Argonaute family of proteins. Argonaute proteins are core components of RNA-induced silencing complexes (RISC) which act in small RNA-directed silencing of target mRNAs. While it is likely that PNH also interacts with small RNAs, it is unclear whether the output of this interaction would be translational repression, cleavage of target mRNAs, methylation of target genes, or other processes. To investigate the role of PNH in leaf polarity and meristem formation, we screened for EMS generated enhancers of the pnh-2 phenotype. To maximise the likelihood of isolating genes involved in the same pathway as PNH, plants were screened for the narrow petal phenotype characteristic of ago/+;pnh/pnh double mutant plants. The initial screen produced 34 enhancer of pinhead (enp) lines. Five of these lines do not exhibit a mutant phenotype in the absence of pnh and were selected for further analysis. The lines can be grouped into 3 phenotypic classes: 1) narrow petals, 2) splayed flowers and 3) ectopic floral organs. Within the recessive class 1 enhancers, double enp1/enp1;pnh/pnh and enp2 pnh/enp2 pnh mutant plants closely resemble ago/+;pnh/pnh mutants, with individual lines differing in phenotypic severity. When doubly mutant with pnh, class 2 enhancers, enp3 and enp4 confer distinctive phenotypes such as splayed floral organs and green and white coloured sepals. The class 3, semi-dominant enhancer, enp5, is the only enp mutation to modify both vegetative and reproductive pnh mutant phenotypes. The vegetative SAM of double pnh enp5/pnh enp5 mutants never terminates in a 'pin' structure and ectopic floral meristems develop in longitudinal rows along the fasciated inflorescence stem. Preliminary data suggests that enp5 is a novel allele of REVOLUTA, an HD-ZIP III family member involved in organ polarity and meristem formation. REV expression is controlled by microRNA regulation, raising the intriguing possibility that PNH influences organ polarity through the control of REV expression. Future experiments will test this possibility and determine the identity of the other enp genes.

180 Elucidating the Role of Basic Helix-Loop-Helix (bHLH) Transcription Factors in Stomatal Development

Naomi Bogenschutz, Lynn Jo Pillitteri, Dan Sloan, Keiko Torii
Department of Biology, University of Washington, Seattle, Washington 98195

Stomata consist of a pair of guard cells, which operate as a turgor-driven valve required for efficient gas and water exchange between a plant and its environment. Stomatal formation begins when an undifferentiated epidermal cell called the meristemoid mother cell (MMC) divides asymmetrically. The smaller daughter cell is called a meristemoid, which renews itself through several rounds of divisions before differentiating into a guard mother cell (GMC). The GMC then divides symmetrically, producing two guard cells surrounding a pore to form a mature stoma. Several cell-signaling molecules have been shown to regulate early steps in stomatal development, including the production of MMCs and the subsequent frequency or orientation of asymmetric divisions. Loss-of-function mutations in TOO MANY MOUTHS (TMM) [1], STOMATAL DENSITY AND DISTRIBUTION (SDD) [2], YODA (YDA) [3], and the ERECTA (ER)-family [4] cause stomata to form next to each other or in clusters, suggesting that these genes transmit cell position and orientation signals. Loss-of-function mutations in genes controlling later steps in stomatal differentiation, such as FOUR LIPS (FLP) [5] and FAMA [3], cause repetitive GMC divisions resulting in stacks of several guard cells, demonstrating their roles in controlling guard cell proliferation. In contrast, no genes have been found that regulate the commitment of a meristemoid to a GMC. By visually screening an ethyl methanesulfonate-mutagenized population of Arabidopsis, we have identified a novel gene, MUTE, that acts as a master regulator for terminal differentiation of the meristemoid to a GMC. MUTE encodes a basic helix-loop-helix (bHLH) transcription factor, where loss-of-function produces a no stomata or "no mouths" phenotype. Unlike wild-type meristemoids that differentiate after 1 to 3 divisions, mute meristemoids divide asymmetrically 3 to 6 times, producing a rosette pattern with an arrested meristemoid at the center. We also determined that a closely related paralog of MUTE is a critical regulator of stomatal development by controlling the initial asymmetric division of the MMC, the first step in the stomatal formation pathway. Characterization of MUTE and its place in the stomata gene regulatory network will be presented.

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181 Active cell-to-cell transport and depletion of Arabidopsis TTG1 determines epidermal trichome patterning

Daniel Bouyer[1], Friedrich Kragler[2], Arp Schnittger[3], Martin Huelskamp[1]

[1]Botany III, University Cologne, Germany, [2]University of Vienna, Department of Biochemistry - Max F. Perutz Laboratories, Austria, [3]Max-Planck-Institut fur Zuchtungsforschung (MPIZ), Cologne, Germany

In plants intercellular communication by moving transcription factors is important for development *(1, 2)*. Epidermal trichome patterning in *Arabidopsis* involves mobile trichome inhibiting MYB-like proteins and trichome promoting factors including the WD40 repeat protein TRANSPARENT TESTA GLABRA1 (TTG1) *(3)*.

Here we demonstrate by clonal analysis using the CRE-LOX system that unexpectedly the trichome promoting factor *TTG1* can act non-cell autonomously. While *TTG1* is expressed ubiquitously TTG1 protein accumulates in trichomes and is depleted in the surrounding cells. The accumulation in trichomes is also seen when using other ubiquitous (CaMV-35S) or even subepidermis-specific promoters.

Microinjection experiments indicate that TTG1 protein actively utilizes plasmodesmata to gain access to neighboring cells. Finally we provide evidence that biasing TTG1 mobility affects patterning.

Taken together our data provide evidence that TTG1 is involved in a substrate-depletion mechanism which accounts for lateral inhibtion of trichome-neighboring cells.

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182 Transcriptional Networks of Plant Stem Cell Control

Wolfgang Busch, Jan Lohmann

Max-Planck-Institute for Developmental Biology

In contrast to animals, plants develop mostly postembryonically and continuously form new organs during their entire life cycle. The cellular basis for this mode of development is the continuous presence of stem-cell pools in the apical meristems of shoot and root, which are the growing points of a plant. The size of the stem-cell pool has to be tightly regulated to avoid ill effects for the organism. In Arabidopsis thaliana, several key factors of stem cell control have previously been identified by genetic approaches. Since most of them are transcription factors, we have set out to elucidate the regulatory network of stem-cell control by means of transcriptional profiling. Focusing on the shoot apical meristem and the floral meristem, we have used loss-of-function mutants, as well as inducible overexpression lines of several key factors including WUSCHEL (WUS), CLAVATA3 (CLV3) and LEAFY (LFY) to identify common and unique targets. By conducting meta-analysis on our expression data and screening for transcripts that follow the genetically defined regulatory logic, such as the negative feedback loop between WUS and CLV3, we were able to identify several high priority targets. Promoter regions of these targets are used for regulatory element searches, with the aim to identify previously unknown sites. Currently we verify the microarray data by quantitative rtPCR and study their spatial expression domains and dynamics by in situ hybridization. Furthermore, we use chromatin immunoprecipitation techniques to study the interaction of the transcription factor WUS with its target genes in vivo. With these diverse approaches we hope not only to gain insight into the in vivo function of target genes, but also into the regulatory logic of stem-cell control. Ultimately, we want to establish a comprehensive model of stem cell homoeostasis with predictive power.

183 Genetic Analysis of Vascular Patterning of the Arabidopsis Root

Annelie Carlsbecker[1, 2], Anne Honkanen[1], Ove Lindgren[1], Siripong Tithamadee[1], Yka Helariutta[1]

[1]Institute of Biotechnology, University of Helsinki, Finland, [2]Dept. of Physiological Botany, Evolutionary Biology Centre, Uppsala University, Sweden

The vasculature of plants is of immense importance as it provides paths for the transport of water, sugars, hormones and other signalling molecules through the phloem and xylem, in addition to providing physical support to the plant through the lignified cell walls of the xylem and fibres associated with the vasculature. Despite its importance, little is known about the regulation of the development of the tissues making up the vasculature. The influence of various hormones have been emphasized by several studies, but few regulatory factors have been identified, and only one, APL, has been shown to determine phloem identity (Bonke et al., 2003, Nature 426:181-186), and the VND6 and 7 genes to determine xylem cell identity (Kubo et al., 2005, Genes & Dev. 19:1855-1860). Furthermore, the genes of the class III HD-ZIP and KANADI families have been shown to influence the patterning of the vasculature in the shoot (Engstrom et al., 2004, Plant Phys. 135:685-694). In order to identify novel components influencing the vascular patterning of the Arabidopsis root, we have performed a genetic screen for mutants miss-expressing the phloem marker AtSUC2::GFP (Imlau A et al., 1999, Plant Cell 11:309-322). This screen resulted in the identification of a set of novel mutants with patterning and/or cell proliferation defects specific to the stele. We named these mutants distorted root vascular pattern1-6 (dva1-6). Collectively, these mutants have short primary roots, display a lack of AtSUC2::GFP expression at the root tip, accompanied by a delayed and distorted phloem development, and develop ectopic xylem in the pericycle along the xylem axis. Interestingly the dva1 and dva2 mutants has a reduced expression of APL, suggesting that they may act upstream of APL. Currently, we are analysing various other cell- or tissue specific markers to characterize theses mutants further and to position them in relation to known factors influencing vascular development.

184 Mutations in the TORNADO2 Gene Enlarge the Peripheral Zone Relative to the Stem Cell Zone in the Shoot Apical Meristem of Arabidopsis thaliana

Wei-Hsin Chiu, John Chandler, Wolfgang Werr

Department of Developmental Biology, University of Cologne, Gyrhofstrasse 17, D-50923 Cologne, Germany

The SHOOTMERISTEMLESS (STM) gene is essential for the correct initiation and maintenance of the shoot apical meristem (SAM). An EMS mutagenesis effector screen performed with the STM:GUS marker line in Arabidopsis thaliana identified a new tornado2 allele, trn2(3010). The histological and genetic analyses implicate TRN2 in SAM function, whereby in trn2(3010) mutants, the peripheral zone is enlarged relative to the central stem cell zone. The trn2(3010) mutant allele partially rescues vegetative stm mutant phenotypes but behaves epistatically to wus1 and clv3-2 alleles during the vegetative phase and in the outer floral whorls. The development of carpels in trn2(3010) wus1 flowers indicates that pluripotent cells persist in floral meristems in the absence of TRN2 function and can be recruited for carpel anlagen. The data implicate a role for a membrane-bound plant tetraspanin protein in cellular decisions in the peripheral zone of the SAM.

185 DAWDLE, a Forkhead-Associated Domain Gene, Regulates Plant Development

David Chevalier, Erin Morris, John Walker
University of Missouri, Columbia, MO

Phosphoprotein-binding domains are found in many different proteins and specify protein-protein interactions critical for signal transduction pathways. Fork-head associated (FHA) domains bind phosphothreonine containing peptides and control many aspects of cell proliferation in yeast and animal cells. The Arabidopsis thaliana protein, Kinase Associated Protein Phosphatase includes a FHA domain that mediates interactions with receptor-like kinases, which in turn regulate a variety of signaling pathways involved plant growth and pathogen responses. Screens for insertional mutations in other Arabidopsis FHA domain containing genes identified a mutant with pleiotropic defects. dawdle (ddl) plants are developmentally delayed, produce defective roots, shoots, flowers, and have reduced seed set. DDL is expressed in the root and shoot meristems and the reduced size of the root apical meristem in ddl plants suggest a role early in organ development.

186 The Circadian Clock Regulates Auxin Signaling and Responses in *Arabidopsis*

Michael Covington, Stacey Harmer
Section of Plant Biology, College of Biological Sciences, University of California, Davis, California, USA

The circadian clock plays a pervasive role in the *temporal* regulation of plant physiology, environmental responsiveness, and development. In contrast, the phytohormone auxin plays a similarly far-reaching role in the *spatial* regulation of plant growth and development. Seventy years ago, Went and Thimann noted that plant sensitivity to auxin varied according to the time of day, an observation which they could not explain. Here we present work that explains this puzzle, demonstrating that the circadian clock regulates auxin signal transduction. Using genome-wide transcriptional profiling, we found many auxin-induced genes are under clock regulation. We verified that endogenous auxin signaling is clock regulated with a luciferase-based assay. Exogenous auxin has only modest effects on the plant clock, but the clock controls plant sensitivity to applied auxin. Significantly, we found both transcriptional and growth responses to exogenous auxin are gated by the clock. Thus the circadian clock regulates some, and perhaps all, auxin responses. As a consequence, many aspects of plant physiology not previously thought to be under circadian control may show time-of-day specific sensitivity, with likely important consequences for plant growth and environmental responses.

The project was supported by the NRI of the USDA CSREES (2004-35100-14903 to MFC) and the NIH (5R01GM069418-02 to SLH).

187 *Arabidopsis BRANCHED* genes are the local switches of axillary bud outgrowth

Jose Aguilar, Cesar Poza, Pilar Cubas
Centro Nacional de Biotecnologia

Plant architecture greatly depends on its branching patterns. Branches are formed from meristems initiated in the axils of leaves. *Axillary* meristems may develop immediately giving new shoots or they may become arrested after a short period of growth as dormant axillary buds. This decision is affected by endogenous and environmental factors. We are studying two Arabidopsis genes coding for TCP transcription factors, *BRANCHED1* (*BRC1*) and *BRANCHED2* (*BRC2*) that control this key decision. *BRC* genes act within the bud, preventing the progression of bud development. They are expressed from the earliest stages of bud development (newly initiated meristems) to the latest stages (reproductive buds) before the bud outgrowth and they become down-regulated at the time of branch elongation. In *brc* mutants, initiation and progression of bud development is accelerated and more axillary buds bolt to give a branch. Consistently to their spatially restricted expression patterns, *brc* mutant phenotypes are not pleiotropic; they affect exclusively bud development. Changes in environmental or endogenous factors affecting bud outgrowth such as growth density or decapitation are associated with changes in *BRC* genes mRNA levels. Moreover, bushy *max* mutants have very reduced levels of *BRC* mRNA and *ycc* mutants, with strong apical dominance, have increased levels of these genes. Therefore *BRC* genes seem to act as local switches of bud growth: they may integrate hormone-mediated developmental and environmental signals controlling bud dormancy and translate them into local responses of axillary growth arrest. *BRC* genes are the orthologs of the *TEOSINTE BRANCHED1* (*TB1*) gene, responsible for the strong apical dominance of the maize suggesting that TB1/*BRC* function is widely conserved among flowering plants. This reveals an ancestral genetic mechanism of branching control that may have evolved in land plants before the emergence of angiosperms. We are currently identyfying the upstream and downstream genes of *BRC* genes to try to reconstruct the cascade of signals controlling branching.

188 Carotenoids and Plant Development

Abby Cuttriss[1], Susan Cossetto[1], Colin Turnbull[2], Barry Pogson[1]
[1]ARC Centre of Excellence in Plant Energy Biology, ANU, Canberra, Australia, [2]Imperial College London, Wye, Kent, United Kingdom

Carotenoid pigments are crucial for the survival and optimal growth of plants. Here we investigate a unique link between carotenoids, apical dominance and chloroplast development in the novel ccr1 (carotenoid and chloroplast regulation) mutant. The ccr1 mutant was characterised in terms of pigment composition, expression of key carotenoid biosynthetic genes and plant morphology. The primary developmental defect in ccr1 was an increase in axillary shoot branching. The primary chloroplastic phenotypes were reduced lutein and aberrant photomorphogenesis. Alterations in the pigment profile were caused by a specific reduction in transcript abundance of the carotenoid isomerase and lycopene ε-cyclase genes. Recent identification of the CCR1 gene and expression analyses indicate that auxin is critical in the control of carotenoid composition and axillary bud outgrowth.

189 Arabidopsis REGULATOR OF AXILLARY MERISTEMS1 Controls a Leaf Axil Stem Cell Niche and Modulates Vegetative Development

Peter Doerner, Thomas Keller
Edinburgh University

Shoot branching is a major determinant of variation in plant stature. Branches, which form secondary growth axes, originate from stem cells activated in leaf axils. The initial steps by which axillary meristems (AM) are specified and their stem cells organized are still poorly understood. We recently reported gain- and loss-of-function alleles at the Arabidopsis REGULATOR OF AXILLARY MERISTEMS 1 (RAX1) locus (Keller et al. 2006). RAX1 is encoded by the Myb-like transcription factor AtMyb37, and is an Arabidopsis homolog of the tomato blind gene. RAX1 is transiently expressed in a small central domain within the boundary zone separating SAM and leaf primordia from early on in leaf primordium development. RAX1 genetically interacts with CUP-SHAPED COTYLEDON (CUC) genes and is required for the expression of CUC2 in the RAX1 expression domain, suggesting that RAX1 acts through CUC2. RAX1 also interacts with other meristem genes, including CLV3. We propose that RAX1 functions to positionally specify a stem cell niche for axillary meristem formation. RAX1 also affects the timing of developmental phase transitions by negatively regulating gibberellic acid levels in the shoot apex. RAX1 thus defines a novel activity that links the specification of AM formation with the modulation of the rate of progression through developmental phases.

Keller, T., J. Abbott, T. Moritz and P. Doerner (2006). Plant Cell 18: 598-611.

190 Class I TCP Genes Link Regulation of Growth and Cell Division Control

Chengxia Li[1], Thomas Potuschak[1], Adan Colon-Carmona[2], Rodrigo Gutierrez[3], Roxana Nadershahi[1], Peter Doerner[1]
[1]Edinburgh University, [2]Salk Institute, San Diego, USA, [3]New York University, New York, USA

During postembryonic plant development, cell division is coupled to cell growth. In active shoot and root meristems there is a stringent requirement to couple these processes, else cell size would be highly variable. In root meristems and during shoot organogenesis cells transit through a zone with high rates of cell growth and proliferation, similar to transient amplification cells in animals. The dynamics of this transition zone implies a need for coordinate regulation of genes underpinning these two fundamental cell functions. We have recently identified a mechanism for co-regulation of cell division control genes and cell growth effectors in proliferating cells (Li et al. 2005). We identified a GCCCR motif necessary and sufficient for the high levels of gene expression normally observed in these target genes. This motif is overrepresented in the promoters of many ribosomal protein genes required for cell growth. The GCCCR motif is required for cyclin CYCB1;1 expression at G2/M and for high-level expression of the S27 and L24 ribosomal subunit genes we examined. We found that class I TCP genes, exemplified by here by the Arabidopsis TCP20 gene, and its product p33TCP20, bind to the GCCCR element in the promoters of cyclin CYCB1;1 and ribosomal protein genes in vitro and in vivo. The expression patterns of the TCP genes we examined are consistent with their proposed role in coordinating high-level gene expression in the transient-amplifying zone of the meristem. We propose a model in which organ growth rates, and possibly shape in aerial organs, are regulated by the balance of positively and negatively acting Teosinte-branched, Cycloidea, PCNA factor (TCP) genes in the proximal meristem boundary zone where cells become mitotically quiescent before expansion and differentiation.

Li, C., T. Potuschak, A. Colon-Carmona, R. A. Gutierrez and P. Doerner (2005). Proc Natl Acad Sci U S A 102: 12978.

191 Regulation Of BDL Gene Expression In The Arabidopsis Embryo

Jasmin Ehrismann, Dolf Weijers, Marika Kientz, Gerd Juergens

Centre for Plant Molecular Biology (ZMBP), Developmental Genetics, University of Tuebingen, Auf der Morgenstelle 3, D-72076 Tuebingen, Germany

Early embryogenesis in Arabidopsis is marked by a series of regulated divisions, that partition the embryo into functionally distinct domains. During this process, different cell types are specified, but the mechanisms are largely unknown. We have previously identified a pair of transcriptional regulators, the activator MP and its inhibitor BDL, that are critical for initation of the embryonic root. Importantly, differential expression of MP and BDL genes is associated with several cell-specification events, including the establishment of apical and basal lineages after the first zygotic division. Hence, identification of cis-elements and transcription factors that regulate MP or BDL expression will shed light on the mechanisms that act in early embryo patterning. We focused our attention on the BDL gene and first generated transcriptional and translational reporter fusions to visualize gene and protein expression, and next preformed performed deletion experiments to narrow down the cis-elements for BDL gene expression to a 50 bp element in the proximal promoter region.. We are currently using yeast 1-hybrid screens to identify transcription factors that bind to this element. We will present the systematic cis-element deletion analysis of the BDL gene and the identification and functional analysis of candidate regulators.

192 Brassinosteroids Regulate Plant Architecture by Controlling Organ Boundaries in Shoot Tissues

Joshua Gendron[1, 2], Dongmei Cao[3], Nathan Gendron[1], Kang Chong[3], Zhi-Yong Wang[1]

[1]Carnegie Institution of Washington at Stanford, Stanford, CA, [2]Stanford University, Stanford, CA, [3]Chinese Academy of Sciences, Beijing, China

Brassinoteroids (BRs) are essential plant hormones that are required for proper development and growth. Much is known about how BRs are made, perceived, and transduced as a signal, and it is well established that BRs play a major role in cell expansion. Little is known about whether or how BRs affect development outside of this role in cell expansion. In this work we show that BRs are involved in establishment of organ boundaries. Organ fusion phenotypes were first noticed in the bzr1-1D mutant, which contains a gain of function mutation in a transcription factor that acts as a positive regulator of BR signaling. bzr1-1D shows kinking of the main stem towards the lateral branches, and BR deficient and insensitive mutants show kinking of the stem away from the lateral branches. Cross sections of the stem-branch junction in bzr1-1D reveal a change in the boundary between the axil cells and the elongating cells of the stem. Additionally, scanning electron microscopy revealed an abnormal fusion of the cauline leaf to the stem. Furthermore, fusions are observed in the bzr1-1D mutant between floral organs within and between whorls. Specifically, bzr1-1D contains stamen-stamen and stamen-carpel fusions, the latter resulting in bent siliques. Brassinolide (BL) treatment of wildtype plants, or induction of an inducible DWF4 biosynthetic gene, leads to stamen-stamen fusion. Additionally, decreased BR signaling leads to abnormal organization of floral organs. A screen for suppressors of the bzr1-1D fusion phenotypes has lead to the identification of intragenic and extragenic suppressors that show no organ fusion phenotypes. GFP tagged versions of the BZR1 wildtype and mutant proteins accumulate in the nuclei of cells in the floral organs demonstrating that BZR1 protein is expressed in the organs that show these fusion defects. Additionally the BZR1 mutant protein accumulates highly in the inflorescence and floral meristems suggesting that it is indeed functioning early in development of the floral organs. Currently we are investigating the effects of BRs on expression of various genes involved in meristem function, including CUC-GFP, CyclinB-GUS, STM-GUS, and others. These results demonstrate that BRs not only determine plant size through regulation of cell expansion but are also involved in developmental patterning and organ separation.

193 Cytokinins regulate both the identity and proliferation of vascular cell lineages

Ana Campilho[1], Melanie Decourteix[1], Ari Pekka Mahonen[1], Anthony Bishopp[1], Kaisa Nieminen[1], Masayuki Higuchi[2], Tatsuo Kakimoto[2], Yka Helariutta[1]

[1]Institute of Biotechnology, University of Helsinki, Finland, [2] Biology Department , Osaka University, Japan

>We are studying the genetic control of vascular morphogenesis during root development in Arabidopsis. The cell lineages which form xylem and phloem and the intervening pluripotent procambial tissue originate from stem cells near the root tip. We have recently shown that cytokinins promote the pluripotent cell identity and inhibit the default protoxylem identity. Either a decrease in cytokinin levels in the procambium, or a disruption of cytokinin-signalling through the two-component phosphorelay (as exemplified by the phenotype of the cre1 ahk2 ahk3 receptor triple knock-out mutant), causes all vascular cells to differentiate into protoxylem cells. AHP6, an inhibitory pseudo phosphotransfer protein, counteracts cytokinin signaling allowing protoxylem formation (Mähönen et al, 2006 Science). On the other hand, we have shown that CRE1/WOL cytokinin receptor is a bifunctional kinase/phosphatase and that replacing CRE1 with AHK2 (with no or low phosphatase activity) results in stimulation of proliferation of vascular cell files (Mähönen, Higuchi et al, in press Current Biology). This indicates that in addition to specifying vascular cell identity, cytokinins have a second role in controlling the rate of proliferation of vascular cell files. Our current progress in understanding which genes control and are regulated by cytokinin action in these two processes will be presented.

194 Gene Expression Programs during Shoot, Root and Callus Development in Arabidopsis Tissue Culture

Ping Che[1], Sonia Lall[1], Dan Nettleton[2], Stephen Howell[1]

[1]Plant Sciences Institute, Iowa State University, Ames IA 50011, USA, [2]Statistics Department, Iowa State University, Ames IA 50011

Shoots can be regenerated from Arabidopsis root explants in tissue culture through a two-step process requiring preincubation on callus induction medium (CIM). Regenerating tissues can be directed along different developmental pathways leading to the formation of shoots, new roots or callus by transferring to shoot- or root-induction medium (SIM or RIM) or continued incubation on CIM. Using gene-profiling methods, we identified genes that were specifically up- or downregulated on one developmental pathway, but not on others. One gene (At5g13330) upregulated during early shoot development encoded an AP2/ERF transcription factor, RAP2.6L. RAP2.6L plays a pivotal role in shoot regeneration because T-DNA knock-down mutations in the gene reduced the efficiency of shoot formation in tissue culture. These same T-DNA mutations reduced the expression of 35% of the genes normally upregulated during SIM incubation, including CUP-SHAPED COTYLEDON 2 (CUC2, At5g53950) a gene involved in shoot meristem specification and required for efficient shoot regeneration. During CIM preincubation, root explants acquire the ability to form green callus or shoots when transferred to SIM. Genes that depend on CIM preincubation for subsequent expression on SIM were identified from expression profiles of explants in which CIM preincubation was omitted. One gene that required CIM preincubation was an A-type response regulator gene, RESPONSE REGULATOR 15 (ARR15, At1g74890). ARR15 appeared to be to a marker for competency to form green callus, because both required only one day preincubation on CIM and were not affected by reversible cell cycle inhibitors. Competence to form shoots requires two or more days of CIM preincubation and can be blocked by reversible inhibitors of cell division.

This work was supported by grants from the National Science Foundation and from the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service.

195 ARROW1 (ARO1), a Novel Regulator of Leaf Polarity in Arabidopsis

Tengbo Huang, Jeon Hong, Randall Kerstetter

Waksman Institute, Rutgers University

Normal leaf morphogenesis requires the proper specification of adaxial-abaxial polarity. Several classes of genes have been demonstrated to control the specification of abaxial and adaxial fate in lateral organs. Among these, KANADI (KAN) genes are essential promoters of abaxial identity. Here, we describe an enhancer of kan that shows significant loss of leaf polarity when combined with kan1 kan2 mutants. This gene we have dubbed ARROW1 (ARO1) because aro1 mutant has arrowhead-shaped leaves. ARO1 encodes a putative sequence-specific RNA-binding protein of the Pumilio/PUF domain family several of which have been shown to function as inhibitors of translation in animals. aro1 plants display pleiotropic developmental defects indicating roles in leaf, root, and flower development. It also interacts with other developmental mutants involved in leaf polarity, such as ASYMMETRIC LEAVES1 (AS1), ASYMMETRIC LEAVES2 (AS2) and REVOLUTA (REV). These features indicate that ARO1 represents a novel regulator of diverse pathways during plant development.

196 The Arabidopsis homeodomain proteins SAW1 and SAW2 control leaf development through negative regulation of KNOX function

Ravi Kumar, Kumuda Kushalappa, Dietmute Godt, Mark Pidkowich, Sandro Pastorelli, Shelley Hepworth, George Haughn

University of British Columbia, Vancouver, BC, Canada

In Arabidopsis, the BEL1–like TALE homeodomain gene family consists of 13 members that form heterodimeric complexes with the Class 1 KNOX TALE homeodomain proteins including STM and BP. The BEL1-like protein BELLRINGER (BLR) functions together with STM and BP in the shoot apex to control meristem identity/function and promote correct shoot architecture. We have characterized two additional BEL1-like genes, *SAWTOOTH1* (*SAW1*) and *SAWTOOTH2* (*SAW2*) that, in contrast to *BLR*, are expressed in lateral organs but not in meristems. Saw1 and saw2 single mutants have no obvious phenotype but the saw1 saw2 double mutant has increased leaf serrations indicating that SAW1 and SAW2 act redundantly to limit leaf margin growth. Consistent with this hypothesis, over-expression of *SAW1* suppresses overall growth of the plant shoot. BP is ectopically expressed in the leaf serrations of saw1 saw2 double mutants. Ectopic expression in leaves of Class 1 KNOX genes has been observed previously in loss-of-function mutants of *AS1*, a gene encoding a MYB transcription factor. For this reason we explored the relationship between SAW1 and BP in an as1 mutant. Over-expression of *SAW1* in an as1 mutant suppresses the as1 leaf phenotype and reduces ectopic *BP* leaf expression suggesting that SAW1 may act downstream of AS1. However, the domain of ectopic expression of *BP* in as1 leaves and the morphology of as1 leaves are distinct from those observed for the saw1 saw2 double mutant. In addition, *SAW1* transcript levels remain unchanged in the leaves of as1 mutant. Taken together, our data suggest that SAW1/SAW2 acts independently of AS1 to establish leaf shape by repressing growth in specific subdomains of the leaf margin at least in part by repressing expression of one or more of the KNOX genes.

197 Cytokinin Receptors are Required for Normal Development of Auxin-transporting Vascular Tissues in the Hypocotyl but not in Adventitious Roots

Takeshi Kuroha[1, 2], Chiharu Ueguchi[3], Hitoshi Sakakibara[2], Shinobu Satoh[1]

[1]Institute of Biological Sciences, University of Tsukuba, Tsukuba 305-8572, Japan, [2]RIKEN Plant Science Center, Tsurumi, Yokohama 230-0045, Japan, [3]Bioscience and Biotechnology Center, Nagoya University, Nagoya 464-8601, Japan

Plants alter the architecture of their root systems to adapt to the environment by modulating post-embryonic (lateral and adventitious) root formation and growth. To better understand the genetic basis of this regulation, we screened ethyl-methane sulfonate-mutagenized lines of *Arabidopsis thaliana* for adventitious rooting mutants. One mutant showed retardation of the primary root growth, no production of lateral roots, and enhanced formation of adventitious roots. Mapping and genetic complementation revealed that this mutant named *wooden leg-3* (*wol-3*) was an allele of *ARABIDOPSIS HISTIDINE KINASE 4* (*AHK4*), a locus known to encode a cytokinin receptor. Although the vascular system of the primary root and hypocotyl in the *wol-3* mutant was aborted, that of the adventitious roots was normally developed. To investigate the relationship between the auxin response and the *wol-3* phenotype, we introduced the auxin responsive promoter construct *DR5::GUS* into wild-type and the *wol-3* mutant. In the hypocotyl of the *wol-3* mutant, strong *DR5::GUS* expression was observed around the aborted vascular system. The *wol-3* primary root shows no *DR5::GUS* expression except in the root tip. When the *wol-3* primary root was cut off, both the expression of *DR5::GUS* and lateral root formation were inhibited. The application of auxin to the *wol-3* primary root resulted in the induction of *DR5::GUS* expression and subsequent lateral root formation, suggesting that the auxin response in the *wol-3* primary root is normal. Furthermore, basipetal movement of radiolabeled IAA transport from the hypocotyl to the primary root in the *wol-3* mutant was significantly inhibited compared to the wild type. Although only a single amino acid alteration had occurred in *AHK4*, the root morphology in the *wol-3* mutant was quite similar to that in the *ahk2 ahk3 ahk4* triple mutant, which is a loss-of-function mutant of the three cytokinin receptors. This implies that the functional disturbance of *AHK4* affects the function of the other receptors. Our results suggest that cytokinin receptors are necessary for the formation of auxin-transporting vascular tissues in the hypocotyl, but not in adventitious roots.

198 SCRAMBLED Mediates Post-embryonic Positional Signaling For Epidermal Patterning In Arabidopsis Roots

Su-Hwan Kwak, John Schiefelbein

Department of Molecular, Cellular and Developmental Biology, University of Michigan

In Arabidopsis roots, epidermal cells have two fates; to be a root hair cell or a non-hair cell. The fate decision is influenced by the position of the epidermal cells. Developing epidermal cells overlying two cortical cells differentiate as hair cells, whereas non-hair cells arise over a single cortical cell. SCRAMBLED (SCM), a leucine-rich repeat receptor-like kinase (LRR-RLK) is required for interpreting the position of developing epidermal cells so that they adopt appropriate fates. It is known that the position-dependent pattern of epidermal cells is initiated during embryogenesis, because *GLABRA2* (*GL2*) expression begins to exhibit a position-dependent pattern within protodermal cells at the heart stage and is maintained throughout the remainder of embryogenesis. We examined the role of SCM in the embryonic patterning. Although a SCM-GFP fusion protein expressed under the SCM promoter is localized in the plasma membrane of embryonic cells, we find that *scm* mutants do not alter the embryonic GL2 expression pattern in protoderm cells. The two closest homologues of SCM, SRE1 and SRE2, in the LRR-V family RLKs, were found to be dispensable for the embryonic patterning. Embryos of sre1-1 sre2-1 double mutant and scm-2 sre1-1 sre2-1 triple mutant still possess the position-dependent GL2 expression pattern. Thus, it appears that SCM and its close homologues SRE1 and SRE2 are not involved in embryonic protodermal patterning, but SCM is involved in the position-dependent epidermal cell fate determination in developing roots at the post-embryonic stage. This may mean that two position-dependent cell fate determination mechanisms may exist; one for the embryonic protoderm and one for the epidermis of post-embryonic developing roots.

199 The Trichome Pock Phenotype of *agb1* and *pom1*

Zachary Larson-Rabin, Christopher Day
University of Wisconsin-Madison

Socket cells, which are specialized epidermal cells surrounding the base of Arabidopsis trichomes, differentiate following an unknown recruitment mechanism. These cells, numbering twelve on average in wild-type Columbia, serve to support the trichome. We have identified two mutants characterized by trichome pocks, which are deep indentations of the leaf surface at the base of some trichomes. The pocks correlate with an increase in socket cell number.

We have mapped one mutant and found that it has a loss-of-function mutation in *AGB1*, the beta subunit of the heterotrimeric G-protein complex. Some of the pleiotropic phenotypes of the *agb1* mutant have been described before, and include auxin and ABA hypersensitivity, *erecta*-like aerial defects, and root morphology and growth alterations. In this poster, we will describe the undocumented trichome pock phenotype of *agb1*.

Another mutant that exhibits the trichome pock phenotype is the *erh2* allele of *POM-POM1 (POM1/ERH2/ELP1/AtCTL1)*. Trichomes of the *erh2* allele show extra socket cells, sometimes as many as 30 per trichome. These plants also have root morphology phenotypes similar to *agb1*. In addition, stomata often form between socket cells or even adjacent to trichomes. This is not seen in wild-type or in *agb1*, in which stomata always form outside the socket cell zone. The gene product of *POM1*, a chitinase-like protein, has been implicated in repressing ethylene production and signaling. Our poster will describe our characterization of the trichome pock phenomenon, with regard to possible intercellular signals that control socket cell number.

200 Adenosine Kinase Deficient Lines Display Abnormal Development

Sarah Schoor[1], Katja Engel[1], Filomena Ng[1], Sanghyun Lee[1], Neil Emery[2], Barb Moffatt[1]
[1]Univeristy of Waterloo, Waterloo Ontario, Canada, [2]Trent Univeristy, Peterborough Ontario, Canada

By recycling adenosine into AMP, adenosine kinase (ADK; EC 2.7.1.20) plays a key role in maintaining nucleotide pools and methylation. In Arabidopsis, ADK is represented by two highly similar isoforms, ADK1 (At3g09820) and ADK2 (At5g03300). Aside from establishing that ADK1 is expressed at higher levels than ADK2 throughout Arabidopsis, no distinct role has been assigned to either isoform. In order to establish the roles of these isoforms, as well as ADK activity itself, gene silencing lines (sADK) and T-DNA mutants lacking either ADK1 or ADK2 were identified. The metabolism and development of these mutants were then examined by establishing their remaining ADK activity and documenting their corresponding growth rate, leaf and meristem development and DNA methylation using HPLC analysis. The results indicate no unique role for either ADK1 or ADK2, since the removal of either isoform results in decreased ADK activity but no discernable phenotypic changes. However, reducing overall levels of ADK activity resulted in severe changes to plant development, with lower ADK activity causing more severe phenotypes. Some of the abnormalities associated with the silencing include: decreased DNA methylation, smaller roots, wrinkled leaves, enlarged inflorescent meristems, clustered inflorescences, delayed leaf and silique senescence and reduced stem development. Secondary shoots that eventually develop on sADK plants are morphologically similar to those of wild-type Arabidopsis (i.e. contain cauline leaves and non-clustered inflorescences). We are using ADK-deficient lines generated by overexpression of ADK-GFP fusion proteins to compare silencing in secondary vs primary shoots. In addition, transformation with a constitutively expressed adenosine deaminase cDNA alleviates the abnormal phenotype of the sADK lines and suggests that increased adenosine is leading to these traits.

201 Phenome-Ready Unimutant Collection of the GRAS Gene Family

Mi-Hyun Lee, Miran Kim, Jung-Ok Heo, Dong Kwan Kim, Sung Oh Sohn, BoHye Kim, Nan-Ie Yu, Chae Eun Lim, Jun Lim

Department of Bioscience and Biotechnology, KonKuk University

GRAS proteins are named after GAI, RGA, and SCR, the first three founding members. Members of the GRAS gene family are predicted to be transcription factors and are involved in various aspects of plant growth and development. In particular, SCRECROW (SCR) and SHOOT-ROOT (SHR) in Arabidopsis regulate asymmetric cell divisions responsible for ground tis sue formation in the shoot and root. In the Arabidopsis genome, 33 GRAS members have been predicted. To study the role of GRAS members in Arabidopsis, we have begun to isolate T-DNA insertion mutant lines for 33 GARS members. As a result, a total of 76 T-DNA insertion lines for 31 GRAS members were isolated by PCR-based genotyping method. Most of the loss-of-function mutants failed to show an obvious visible phenotype, although mutations in the published members exhibited corresponding phenotypes as previously reported. These observations suggest that the GRAS members encode similar proteins performing overlapping functions during plant growth and development. Since no obvious phenotype was visible in most of the mutants, we have generated over-expression lines for the members as well as multiple (double, triple or quadruple) mutants. Construction of over-expression lines was facilitated by Gateway-compatible binary vector system.

In addition, we performed real-time quantitative RT-PCR (qRT-PCR) and yeast two-hybrid (Y2H) screening to investigate the interactions among 33 GRAS members. Current progress in the completion of phenome-ready unimutant collection of the GRAS family will be presented.

202 Double Mutant Analyses Reveal Functional Interactions Between MDR (PGP) ABC-Transporter Genes and also Endogenous Regulators of Auxin Transport

Daniel Lewis, Nathan Miller, Edgar Spalding

University of Wisconsin-Madison

In roots, auxin moves toward the apex (acropetally) in central cells and towards the base (basipetally) in outer cells. Mutations in *MDR1* reduce acropetal auxin transport by 80% without affecting basipetal transport. Conversely, loss of *MDR4* reduced basipetal auxin transport by 50% without an effect on acropetal transport. Thus, these two mutations allow the influences of acropetal and basipetal auxin flows to be separated. Previosly described differential growth phenotypes were re-analyzed with a new version of morphometric analysis software capable of judging statistical signifigance. In order to test the interdependence of acropetal and basipetal auxin transport on root development, the *mdr1mdr4* double mutant was subjected to gravitropic and vertical growth assays. The results show that these two transport streams act independently to modulate separate differential growth responses. Double mutant analysis was also employed to address the functional relationship between MDR4 and flavanoids capable of inhibiting auxin transport. By combining *tt4*, a mutation that blocks flavaonid synthesis, increases basipetal auxin transport, and exhibits a slower gravitropic response with *mdr4*, we were able to use analysis of the gravitropic response as a means of testing weather flavanoids act through inhibiting MDR function in vivo as well as in heterologous systems. Preliminary results indicate the double mutant is hypertropic compared to *tt4* and becomes signifigantly more curved than wild-type after 3 hours of gravistimulation. While these data alone do not demonstrate an epistatic relationship due to the kinetic differences between *mdr4* and *mdr4tt4*, they support the postulation that flavaniods at least partially affect basipetal auxin transport through MDR4.

203 Control of Leaf Vascular Patterning by Polar Auxin Transport

Enrico Scarpella[1], Danielle Marcos[2], Jiri Friml[3], Thomas Berleth[2]

[1]Department of Biological Sciences, University of Alberta, CW-405 Biological Sciences Building, Edmonton AB, T6G 2E9 Canada, [2]Department of Botany, University of Toronto, 25 Willcocks Street, Toronto ON, M5S 3B2 Canada, [3]Center for Plant Molecular Biology, Tubingen University, D-72076 Tubingen, Germany

The formation of the leaf vascular pattern has fascinated biologists for centuries. In the early leaf primordium, complex networks of procambial cells emerge from homogeneous subepidermal tissue. The molecular nature of the underlying positional information is unknown, but various lines of evidence implicate gradually restricted transport routes of the plant hormone auxin in defining sites of procambium formation. Here we show that a crucial member of the Arabidopsis AtPIN family of auxin efflux associated proteins, AtPIN1, is expressed prior to preprocambial and procambial cell fate markers in domains that become restricted towards sites of procambium formation. Subcellular PIN1 polarity indicates that auxin is directed to distinct convergence points in the epidermis, from where it defines the positions of major veins. Integrated polarities in all emerging veins indicate auxin drainage towards pre-existing veins, but veins display divergent polarities as they become connected at both ends. Auxin application and transport inhibition reveals that convergence point positioning and PIN1 expression domain dynamics are self-organizing, auxin-transport dependent processes. We derive a model for self-regulated, reiterative patterning of all vein orders and postulate at its onset a common epidermal auxin-focusing mechanism for major-vein positioning and phyllotactic patterning.

204 Automated phenotyping of Arabidopsis root and shoot structures responding to light and gravity stimulation

Nathan Miller[2, 3], Edgar Spalding[2]

[2]Department of Botany, University of Wisconsin, [3]Doctorate program in Biomedical Engineering

Technologies for finding and quantifying phenotypes have not kept pace with those for producing mutants and other genetic resources. In an effort to correct this imbalance, we have been developing software and analytical methods that will support a high-throughput, automated platform for detailed analysis of plant morphologies and developmental processes. Our approach begins with high-resolution electronic images of plant structures undergoing developmental responses to changes in an environmental variable. One example with which we have been successful is the etiolated seedling responding to first light. Another is the seedling root responding to gravistimulation. Images taken every two to ten minutes are processed using custom computer algorithms to isolate the midline of the shoot or root at each time point because the midline contains the key invariant shape information. From a developmental series of midlines, length, axial curvature distribution, and rates of change in these parameters are computed. These shape parameters are sufficient to describe the growth and development of form of these structures. The spatiotemporal resolution of the method is such that phenotypic differences that escape detection by eye can be rigorously quantified. Examples of how the technology has aided studies of specific photomorphogenic mutants responding to light and auxin transport mutants responding to gravity will be presented, as will be our progress toward making a phenotype-screening platform. Methods from the field of statistical learning are being employed to identify and categorize individuals that display non-wild-type responses. A recent test shows that our automatic method correctly classifies a mutant that displays a very subtle curvature phenotype. As more data are collected, the discerning power, the ability to tell mutant from wild type, will increase. A near-term goal is to screen segregating populations of T-DNA tagged knockout mutants to identify those with subtle phenotypes without first having to isolate homozygous individuals. The ultimate goal is to integrate 'morpholome' data with expression array and other 'omic' level data to create a full systems level view of Arabidopsis development.

205 Identification of Genes Implicated in Arabidopsis Root Patterning using a GAL4/UAS Activation Tagging System

Shunsuke Miyashima, Takamitsu Waki, Takashi Hashimoto, Keiji Nakajima
Grad. School Biol. Sci., NAIST

The *Arabidopsis* root possesses the highly organized cell pattern. This cell pattern is formed initially during embryogenesis. After germination, stem cells in the root meristem commence stereotyped cell division sequences that perpetuate the cell pattern. Until now, a few recessive mutants that are defective in the root patterning have been isolated, and their causal genes were identified. However it is becoming more difficult to identify novel genes that act in root patterning by simple mutant screening, due to genetic redundancy. To identify novel genes involved in the root patterning, we have developed a new activation tagging system, in which *Arabidopsis* plants expressing a GAL4:VP16 transcription activator in a tissue-specific manner were transformed with a T-DNA containing GAL4-binding sequences (UAS). We screened approximately 17,200 transgenic lines and isolated eight dominant mutants designated *UAS-tagged root patterning (urp)*, *urp1D* through *urp8D*. Genes tagged by the UAS were identified by determining T-DNA insertion sites, followed by co-segregation and recapitulation experiments. Seven of the eight genes were found to encode putative transcription factors belonging to the AP2, NAC, C2H2 Zinc finger, R2R3 MYB, DOF and RWP-RK families. Expression studies indicated that some of these genes are expressed specifically in certain cell types in the root meristem region. These observations indicated that the GAL4/UAS activation tagging system is a useful tool to identify novel genes that have escaped from conventional mutant screenings. *Arabidopsis*

206 Analysis of vesicle transport system governing vascular continuity

Satoshi Naramoto[1], Schinichiro Sawa[1], Koji Koizumi[2], Takashi Ueda[1], Akihiko Nakano[1, 3], Hiroo Fukuda[1]
[1]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, [2]Department of Botany, University of Toronto, [3]Molecular Membrane Biology Laboratory, RIKEN

Within the leaf of an angiosperm, the vascular system is constructed in a complex network pattern. The formation of this vein pattern has been studied as a paradigm of tissue pattern formation in plants. In order to elucidate the molecular mechanism controlling venation pattern, van1 to van7 mutants of Arabidopsis, showing a discontinuous venation, were isolated. In this session, we report a molecular mechanism governing vascular continuity through the analysis of the van3 and van4 mutants that have more preferential defects in vein continuity than the other van mutants.

At first, we characterized VAN3 gene that encoded an ARF-GAP protein. ARF-GAP is known to be a regulator of budding of vesicles in intracellular compartments. Therefore, we analyzed the subcellular localization of VAN3 with transgenic plants and suspension cells expressing both the VENUS tagged VAN3 and a GFP tagged individual organelle markers. These analyses showed that VAN3 was localized not only on a subpopulation of TGN and unknown organelles. Interestingly, VAN3 was not distributed uniformly on TGN but formed a distinct domain. Taken together with the finding that VAN3 was primarily localized in Triton X-100-insoluble fractions of microsome membranes, VAN3 may reside on a raft-like domain in TGNs.

Next, toward characterizing the function of plant ACAPs, subcellular localization analysis of VAN3 like (VAL) proteins was performed. This analysis showed that VAL1 and VAL2 were localized on endosomes, whereas VAL3 was localized in the cytoplasm. Expression pattern analysis of VAN3 and VALs revealed that these genes were expressed in distinctive developmental processes. These findings suggested that plant ACAPs were functionally differentiated.

Finally, the VAN4 gene was characterized. VAN4 encodes a novel protein having no clear homology to any gene of known functions. However, subcellular localization analysis revealed that VAN4 resided on endosomes, suggesting its involvement of endocytosis. The polarization of PIN1 was widely known to play a critical role in determining the venation pattern. However, based on the venation pattern of van3pin1 and van4pin1 double mutants, we found that PIN1 functions independently from VAN3 and VAN4. Based on these results, we discussed a venation pattern formation mechanism from the view of vesicle transport.

207 FAMA Controls The Switch Between Proliferation And Differentiation In Stomatal Development.

Kyoko Ohashi-Ito, Dominique Bergmann
Department of Biological Sciences, Stanford University

Stomata are essential for the exchange of gases and water vapor between a plant and its environment. Although previous studies revealed several factors involved in the signaling that creates normal stomatal pattern, less is known about the positive regulators of stomatal differentiation. We are studying *FAMA*, a bHLH transcription factor, which is highly expressed in plants having excess stomata and repressed in plants without stomata. A T-DNA insertion line of *FAMA* (*fama-1*) has no morphologically identifiable stomata in any organ. Instead, *fama-1* mutants make tumor-like clusters in normal stomatal positions. We have shown that the cells in these tumors express markers of developing stomata, but do not express mature stomatal markers. FAMA RNA and protein are expressed in specific cells of the stomatal lineage. Overexpression of *FAMA* leads to a phenotype in epidermal cells that is the opposite of the loss of function phenotypes. *FAMA*-OE plants make many ectopic unpaired-guard cells. These results suggest that FAMA's function is to promote differentiation of guard cells and to inhibit excess cell divisions in stomatal development. Further studies on the activity of FAMA and its interaction with other stomatal regulators will be presented.

208 Analysis of ARF7- and ARF19- Regulated Genes in *Arabidopsis* Lateral Root Formation

Yoko Okushima[1], Makoto Onoda[1], Athanasios Theologis[2], Masao Tasaka[1], Hidehiro Fukaki[1]
[1]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan, [2]Plant Gene Expression Center, UC Berkeley, Albany, CA

The members of Auxin Response Factor (ARF) regulate auxin-mediated gene expression during plant growth and development. *arf7 arf19* double mutants exhibit strong auxin-related phenotypes including severely impaired lateral root formation, which not seen in each single mutant, suggesting that lateral root formation is redundantly regulated by ARF7 and ARF19 transcription factors. Global gene expression analysis has revealed that auxin-induced expression of many genes, such as those encoding Lateral Organ Boundaries (LOB) domain (LBD)/AS2-like (ASL) proteins, are disrupted in *arf7 arf19* double mutants. Among these auxin-responsive *LBD* genes, *LBD16* and *LBD29* are specifically expressed in root steles and young lateral root primodia, where *ARF7* and *ARF19* are also expressed. We found that overexpression of *LBD16* and *LBD29* partially rescues the lateral root phenotype of *arf7 arf19* double mutant, respectively. In addition, target-gene analysis using the *arf7 arf19* plants expressing ARF7-GR fusion protein indicates that ARF7 directly regulates auxin-mediated gene expression of *LBD16* and *LBD29*. These observations strongly suggest that at least ARF7 promotes lateral root formation through the direct activation of auxin-responsive *LBD16/29* expression. Furthermore, the transgenic plants overexpressing LBD16 with transcriptional repression domain (SRDX) exhibit a strong auxin-related phenotype including impaired lateral root formation. Together, our data strongly suggest that auxin-mediated induction of these *LBD*s by ARF7/19 promotes lateral root formation in *Arabidopsis*.

209 Resolving The Function of TAS3-Generated ta-siRNA And ASYMMETRIC LEAVES2 (AS2) in AUXIN RESPONSE FACTORS (ARFs) 2/3/4 Regulation

Irena Pekker, John Alvarez, Moti Sabban, Yuval Eshed
Weizmann Institute of Science, Israel

ARF3/ETT and its closest homolog, ARF4, are essential components of the KAN signaling pathway, which specifies abaxial fate early in organ development. Recently three ARF genes, ARF2, ARF3 and ARF4 were shown to be a common target of ta-siRNAs derived from the TAS3 gene. Simultaneous downregulation of ARFs 2/3/4 significantly enhanced the ett-1 arf4-1 double mutant phenotype, demonstrating that ARF2 carries redundant functions with ARF3 and ARF4 in establishing the abaxial fate of lateral organs. In-situ hybridization with LNA-modified DNA probe revealed an adaxial expression pattern of TAS3-generated ta-siRNA in wild-type Arabidopsis seedlings. To evaluate the in vivo consequences of disrupting ta-siRNA regulation, we constructed a ta-siRNA-resistant version of ARF3 (ARF3R). By sharp contrast to nearly normal plants expressing ectopic ARF3, plants expressing ANT>>ARF3R gave rise to two to three first radial leaves, with gradual shift to expanding asymmetric leaves with adaxial outgrowths, indicating that posttranscriptional ARFs regulation is crucial for organ asymmetry patterning and morphogenesis. ASYMMETRIC LEAVES1 (AS1) and AS2 genes are also responsible for establishing leaf asymmetry by specifying leaf adaxial identity. It was shown that ectopic expression of AS2 in the leaves resulted in the replacement of the abaxial cell types with adaxial ones in a way that mimics the kan1-2 kan2-1 and ett-1 arf4-1 double mutant plants. However, ectopic KAN couldnt complement the asymmetry disruptions caused by AS2 overexpression. In contrast, ectopic ETT could partially complement the ectopic AS2 phenotype. Moreover, as2 mutation enhances 35S:ETT phenotype causing development of lotus-like leaves and adaxial outgrowths, suggesting that AS2 may act as a negative regulator of ETT and ARF4. To distinguish between possible posttranscriptional and translational levels of AS2 regulation we applied inducible protein expression system where AS2 was translationaly fused with Glucocorticoid Receptor (GR). We could show that 12 hours following single DEX application AS2 leads to significant downregulation of ETT and ARF4 transcripts. We are currently trying to figure out whether AS2 mediated ARFs transcripts decay is caused by induction of TAS3-generated ta-siRNA cleavage.

210 Indole-3- acetic acid and its Chemical Analogue 2,4-dichlorophenoxyacetic acid Evoke Differential Responses in Arabidopsis Root Growth

Abidur Rahman[1], Yutaka Oono[2], Elison Blancaflor[3], Tobias Baskin[1]
[1]University of Massachusetts, Amherst, MA, [2]Japan Atomic Enrgy Association, Takasaki, Japan, [3]Noble Foundation, Ardmore, OK

Auxin plays a pivotal role in controlling plant development from embryogenesis to senescence. Although auxin action is becoming well understood, a gap concerns the relationship between the action of the major native auxin, indole-3-acetic acid (IAA) and that of the synthetic auxin, 2,4-dichlorophenoxy-acetic acid (2,4-D), which is used extensively as a source of auxin because of its greater stability. In an effort to fill the gap, we have recently characterized an arabidopsis mutant aar1, which shows a specific resistance to 2,4-D, but exhibits a wild-type response to natural auxins, IAA, indole-3-butyric acid (IBA) and 1-naptheleneacetic acid (NAA), suggesting that the 2,4-D and IAA response pathway is at least partially distinct. To further illuminate the differences between these two growth regulators we took both physiological and cell biological approaches to investigate the downstream events in arabidopsis root growth. The mechanism of auxin-induced growth inhibition was investigated by measuring rates of root elongation and of cell production. At a concentration inhibiting elongation rate by 50%, IAA had no significant effect on cell production rate whereas 2,4-D reduced it dramatically. The other auxins such as IBA and NAA acted more like IAA. The differential effect of IAA and 2,4-D on cell production was further supported by the expression analysis of M-phase reporter, Cyc1B::GUS, which was inhibited by 2,4-D but not by IAA. A link between cytoskeletal organization and the auxin-induced inhibition of cell production and cell elongation was established by monitoring the actin organization in the roots treated with these growth regulators. Whereas IAA appeared to bundle the actin filaments, 2,4-D degraded the actin filaments extensively, mimicking the effect of a bona fide actin inhibitor, latrunculin B (Lat B). Additionally, Lat B mimicked the 2,4-D effect on root elongation and cell production rate. Taken together these results suggest that 2,4-D inhibitis cell production via the actin cytoskeleton whereas the machinery for cell elongation is most sensitive to IAA.

211 Subtilisin-like serine proteases are involved in a broad range of developmental and physiological processes in Arabidopsis thaliana

Carsten Rautengarten, Dirk Buessis, Thomas Altmann
University of Potsdam

The Arabidopsis thaliana genome contains 56 genes encoding subtilisin-like serine proteases (AtSBTs). To gain insight into the roles of the Arabidopsis subtilases we started a systematic functional genomics analysis program. It covers detailed sequence and expression analyses on transcript and protein level, the collection and evaluation of knockout mutants for the entire subtilase gene family, generation of multiple knockouts / knockdowns as well as overexpression of selected subtilase genes. Whereas the mutation of almost any single member of the family did not result in any informative phenotype under standard cultivation conditions, different approaches uncovered important key players in plant developmental and physiological processes. Phenotypic analyses identified an Arabidopsis subtilase to be involved in mucilage extrusion and hpRNA mediated silencing of closely related family members uncovered AtSBTs that act redundantly in senescence associated processes. Ectopic expression identified an AtSBT to be involved in lateral root initiation a hitherto poorly understood process. Proteomic analyses identified another AtSBT probably involved in the initiation of seed storage protein degradation. Our current results emphasize the importance of subtilisin-like serine proteases in plant growth and development.

212 The spd Mutants of Arabidopsis Disrupt Plastid Development in Embryo-Derived Cells

Nicholas Ruppel, Roger Hangarter
Indiana University

In plants, plastid development in seedling tissues can be critical for survival to adulthood. During germination, plastids will develop to perform critical functions such as photosynthesis (chloroplasts) and gravity perception (amyloplasts). We have identified a novel class of mutants in Arabidopsis that affect plastid development upon germination, specifically in the embryo-derived tissues of the seedling. The mutants exhibit cotyledon and hypocotyl albinism upon germination due to improper chloroplast development, whereas tissues derived from the shoot apical meristem are green and appear to develop normal chloroplasts. Since the phenotype of these mutants is seen specifically in seedling tissues derived from the embryo, they have been termed *s*eedling *p*lastid *d*efective (*spd)* mutants. In this report, we describe the characterization of *spd2*. When *spd2* embryos are permitted to fully mature, less than 20% of seedlings survive to adulthood in the absence of a supplemental carbon source. The *SPD2* gene has been cloned and shown to encode for a plastid-targeted elongation factor-G (EF-G). In prokaryotes, the EF-G is a critical component of the translation apparatus. SPD2 may therefore serve a similarly important role in chloroplast translation. We have found that expression of SPD2 in a temperature-sensitive *E. coli* EF-G mutant (fusA101) can rescue the wild-type phenotype, thus showing that SPD2 serves as a functional EF-G. The EMS mutation that causes the *spd2* phenotype is the result of a G to A base change, which converts a conserved glycine residue to an arginine between the P-loop and Switch I regions of the GTP-binding domain found in elongation factors. The amino acid change in *spd2* may therefore disrupt the GTPase activity of the protein. Based on its mutant phenotype and the role of EF-Gs in prokaryotes, the biological activity of SPD2 is most likely required for plastid protein translation during late stages of embryogenesis and/or during germination. In summary, the *spd2* mutant represents a unique resource for studying critical, but poorly understood, aspects of plastid development that are an integral part of embryo maturation and seedling establishment.

213 Identification of Genes Expressed During the Early Root Vascular Development in *Arabidopsis*

Ayako Sakai, Tomoyuki Sasaki, Ryuji Tsugeki, Kiyotaka Okada
Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Japan

In *Arabidopsis*, the root vasculature contains two files of protoxylem and of protophloem elements, which are alternately located on the edge of the vascular cylinder. Metaxylem develops centripetally from the protoxylem poles, creating the continuous xylem plate. Metaphloem develops from cells interiorly adjacent to protophloem cell files. Consequently, phloem is separated into two regions by the xylem plate. Mechanisms of formation of this xylem-phloem pattern in the vasculature are not well understood.

As a first step to clarify the mechanisms of the xylem-phloem patterning and the development, we carried out enhancer-trap screening for genes expressed in the early stages of root vascular development. We obtained 376 enhancer-trap lines expressing reporter GFP in the procambium and/or vasculature. In 16 lines, GFP was expressed in vascular initial cells and their daughter cells. In order to identify genes responsible for the expression of GFP, we performed *in situ* hybridization analysis using probes of genes flanking insertion sites of enhancer-trap T-DNAs. So far, we identified the following 4 genes expressed in vascular initial cells and their daughter cells in cell-type-specific manner. Identified 4 genes are 1) a novel gene expressed only in one or two cell files at the center of vasculature, 2) *IAA* gene expressed in the metaxylem and its adjacent cell files at the center of vasculature, 3) a gene encoding heavy-metal-associated protein expressed in cell files of the xylem and protophloem, and 4) bHLH gene expressed in the cell files neighboring the protoxylem and protophloem. The former three genes (1~3) are expressed in vascular initial cells as well as in their daughter cells. The bHLH gene (4) is not expressed in the vascular initial cells, but expressed in their daughter cells. Expression patterns of the aforementioned enhancer-trap lines and genes will be presented.

214 12 Amino Acid CLV3 Peptide Is Necessary and Enough For Its Function In Arabidopsis SAM and RAM

Shinichiro Sawa, Atsuko Kinoshita, Hiroo Fukuda
The University of Tokyo, Graduate school of Science

The Arabidopsis CLV3 gene encodes a stem cell-specific small protein presumed to be a precursor of an uncharacterized secretory peptide. In order to determine the functional minimum CLV3 peptide, we chemically synthesized various length of CLV3 peptide with two hydroxy proline. We found that the 12 amino acid, MCLV3, is necessary and enough for the CLV3 function to trigger the consumption of the shoot and root meristem. MCLV3 application induced reduced shoot apical meristem size. In about 5% plants, a leaf like structure was produced at the top of the SAM. Even in this case, all of the leaves still have an abaxial-adaxial polarity. In the MCLV3 treated SAM, reporter gene expression levels of CLV3::GUS and WUS::GUS were down regulated. Furthermore, reporter gene activity of the CLV3::GFP was diminished in 2 days after MCLV3 application. In order to examine that the MCLV3 function in the CLVATA pathway, we treated the clv1, clv2, clv3 and some other mutants with the MCLV3. MCLV3 application did not affect to the SAM of clv1, clv2, sol1, and sol2, but it reduced the SAM size of clv3 and WT. On the other hand, MCLV3 did not affect to the root meristem of clv2 and sol2, and it induced short root phenotype to clv1, clv3, and sol1 mutant. These results indicated that the MCLV3 function in the CLAVATA pathway, and its ligand-receptor combination may be different between SAM and RAM.

In Arabidopsis genome, 30 CLE genes were reported, and we found one more CLE gene, named CLE46. The application to Arabidopsis, Rice, and Physcomitrella of 26 different synthetic dodeca-peptides corresponding to all of the 31 Arabidopsis CLE genes, revealed diverse signaling pathways involved in cell fate of stem cells. Here we will discuss about the CLV3 and other CLE gene function and molecular mechanisms in higher plants.

215 TRIDENT and VARICOSE encode components of the RNA decay machinery and are required for normal leaf blade expansion

Leslie Sieburth, David Goeres, MaryLou Spencer, Weiping Zhang, Jaimie Van Norman
University of Utah

To understand the molecular basis for leaf blade development, we are characterizing mutants with leaf blade (and vein patterning) defects. One of our mutants, trident (tdt), develops into very small sickly seedlings with multiple vascular defects. In contrast to the wild type, which produces cotyledon veins composed of two to four complete areoles, tdt cotyledons have no complete aeroles, and instead produced fork-like vein patterns. In addition, tdt mutants also have vascular defects in the transition zone (hypocotyl apex) and occasional ectopic tracheary elements within the cotyledon lamina. Aspects of the tdt phenotype are similar to that of varicose (vcs), a mutant that we characterized previously (Deyholos et al., 2003 Devel 130:6577). To determine the molecular basis for the tdt phenotype, we mapped the gene to chromosome 5. One candidate gene within our mapping interval contained a 50 nt deletion (in At5g13570). To confirm that this was the gene that caused the tdt phenotype, we obtained a SALK insertion allele (exon 3) for this gene. The salk allele (tdt-2) failed to complement the tdt-1 allele, indicating that the two lines have mutations in the same gene. The TDT gene encodes a putative protein with two well-conserved N-terminal domains, a DCP2 domain, and an adjacent NUDIX domain. This gene identity indicates that TDT encodes an mRNA decapping enzyme. VCS encodes a putative protein with an N-terminal proline-rich domain followed by two WD domains, and shows similarity to the human RCD8 autoantigen. Recently, RCD8 was renamed GE-1/HEDLS, and was shown to be a component of P-Bodies and to bind hDCP2 and hDCP1 (Yu et al., 2005 RNA 11:1795; Fenger-Gron 2005 Mol Cell 20:905). The phenotypic similarity of vcs to tdt, and the molecular interaction between hVCS/HEDLS/GE-1 and hDCP2 suggests that VCS functions with TDT in carrying out RNA decay.

216 Characterization of LcrTFL1, the Leavenworthia crassa TERMINAL FLOWER 1 ortholog

Marek Sliwinski, David Baum
University of Wisconsin Madison

TERMINAL FLOWER 1 (TFL1) encodes a small protein that is expressed in Arabidopsis inflorescence meristems (IM) and functions to prevent the IM from adopting a floral fate by suppression of the floral meristem identity gene LEAFY (LFY). tfl1 plants flower early and produce a determinate inflorescence in which the apical meristem is converted to a terminal flower soon after the transition to flowering. In contrast, 35S::TFL1 lines are delayed in flowering and the number of vegetative nodes increases 2-3 fold. These lines also have an increased number of paraclades, secondary shoots with cauline leaves (I1) and novel secondary shoots without a subtending leaf (I1*). TFL1 and LFY homologs have been identified in a number of species and have been postulated to play a role in the evolutionary diversification of shoot architecture. Prior work with Leavenworthia crassa (Lcr) which produces flowers on long pedicels directly from the rosette, showed that the LcrLFY gene differs from LFY in the regulation of TFL1. To test the hypothesis that there has been molecular coevolution of LcrTFL1 and LcrLFY, and this played a role in the evolution of rosette flowering, we cloned and characterized LcrTFL1 including introns and cis-regulatory regions. LcrTFL1 is able to rescue the premature production of terminal flowers in tfl1 mutants indicating LcrTFL1 protein function and cis-regulation are largely conserved. LcrTFL1 transgenic plants did differ from wildtype in other aspects of inflorescence architecture. As in 35S::TFL1 lines, LcrTFL1 causes an increase in the number of paraclades, yet unlike 35S::TFL1 this is predominantly in the form of more I1 paraclades rather than I1* paraclades. This increase is dominant (it also occurs in LcrTFL1;TFL1 plants) suggesting LcrTFL1 is more potent than TFL1 in maintaining the I1 developmental phase or less potent at suppressing bract production. In addition, LcrTFL1 is different from 35S::TFL1 lines in that flowering time is not delayed. LcrTFL1 does not extend the vegetative phase suggesting LcrTFL1 expression before the transition to flowering is repressed. We also rescued tfl1 using an EGFP:LcrTFL1 translational fusion construct allowing us to explore protein expression driven by the LcrTFL1 cis-regulatory regions in an Arabidopsis genetic background.

217 Regulation of inflorescence architecture in Arabidopsis

Harley Smith, Siddhartha Kanrar, Ricardo Junqueira, Moni Bhattacharya

Center for Plant Cell Biology, Dept. Botany and Plant Sciences, University of California, Riverside

Flowering is a major developmental phase change that transforms the fate of the shoot apical meristem (SAM) from a leaf bearing vegetative meristem to that of a flower producing inflorescence meristem. In Arabidopsis, specifying flowers and internodes involves the integration of environmental and endogenous floral promoting cues at the SAM. To date, little is known about the molecular mechanisms that integrate the floral inductive cues to specify the inflorescence pattern of growth. In Arabidopsis, two redundant functioning homeobox genes, PENNYWISE (PNY) and POUND-FOOLISH (PNF), which are expressed in the vegetative and inflorescence SAM display a non-flowering phenotype during inflorescence development. In addition, internode patterning is severely impaired in pny pnf plants. At the biochemical level, PNY and PNF interact with another homeobox protein, SHOOTMERISTEMLESS (STM). Weak alleles of stm display an inflorescence phenotype that is comparable to pny pnf double mutants, indicating that PNY-STM and PNF-STM regulate inflorescence architecture. Genetic studies from our laboratory indicate that PNY-STM and PNF-STM heterodimers function as co-factors for transcriptional complexes that regulate floral specification, internode patterning and the maintenance of boundaries between initiating floral primordia and the inflorescence meristem.

218 Properties of Subtilase Genes Associated with Plant Development in Arabidopsis

Jianxiang Liu, Renu Srivastava, Sudhansu Dash, Ping Che, Stephen Howell

Plant Sciences Institute, Iowa State University

Subtilisin serine proteases (subtilases) are encoded by a family of 56 genes in Arabidopsis (Rautengarten et al., 2005). We became interested in these genes when three of them (At1g01900, At4g26330 and At5g59120) appeared on a list of genes upregulated in association with a major QTL conditioning shoot development in Arabidopsis (Lall et al., 2004). We were awarded a NSF 2010 grant to understand the function of these three subtilase genes and seven others (At5g51750, At1g32960, At1g32940, At4g21326, At5g59090, At1g20160, At5g59810 and At5g45650) that are regulated during shoot, root or callus regeneration in culture. We are also part of a The Arabidopsis Subtilase Consortium (TASC, http://csbdb.mpimp-golm.mpg.de/csbdb/dbcawp/psdb.html) that seeks to understand the function of all plant subtilase genes. Many of the subtilase knockouts do not have obvious phenotypes (Rautengarten et al, 2005), hence, functional studies are being directed toward understanding the properties of the genes and their gene products. Expression profiling during organ regeneration revealed that At1g01900 is upregulated both during callus and shoot development. Two of the genes (At1g32940 and At5g59810) are highly upregulated when root explants are placed on auxin-rich callus induction medium. At4g21326, At5g59090 and At5g45650 are upregulated during shoot development and At5g51750, At4g26330 and At1g20160 are upregulated during root regeneration. The localization of subtilase gene expression based RT-PCR analysis has been reported by Rautengarten et al. 2005. We expanded on that analysis using promoter: GUS constructs and demonstrated that At1g01900 is expressed in newly formed callus and shoot primordia during organ regeneration in culture. Many of the subtilase genes showed a strong disposition for expression in guard cells, such as At1g01900, At1g32960, At5g51750, At5g59090, At5g59120 and At5g59810 while others such as At4g21326 and At1g20160, were expressed in veins. Most of the subtilases in our study encode preproenzymes, which are predicted to be secreted proteins. The exceptions are At4g21326 and At4g26330, which lack perceptible presequences. YFP-fusion constructs demonstrated that, for example, the product of At1g01900 accumulates in the plasma membrane or apoplast. The subtilases are being expressed in heterologous systems or as tap-tag constructs in Arabidopsis with the aim of characterizing the enzymatic properties of the protein and the nature of the substrates.

219 Analysis of a Mutant, *#1-63*, which Exhibit an Abnormal Expression Pattern of *FILAMENTOUS FLOWER*

Koichi Toyokura[2], Keiro Watanabe[1], Noritaka Matsumoto[2], Kiyotaka Okada[2, 1]

[1]CREST, JST, [2]Department of Botany, Graduate School of Science, Kyoto University

In *Arabidopsis thaliana*, adaxial side (upper side) and abaxial side (lower side) of leaves have different characteristics, e.g. shapes of cells and number of trichomes. *FILAMENTOUS FLOWER* (*FIL*) is known to be expressed on the abaxial side. When *FIL* is overexpressed under the control of CaMV 35S promoter, adaxial sides of the leaves are abaxialized, and in severe case filamentous leaves with only abaxial characters are produced. These suggest that normal expression pattern of *FIL* is required for establishing adaxial and abaxial properties. In order to understand the mechanisms of controlling the ab-adaxiality, it is crucial to reveal the mechanisms of the regulation of *FIL* expression.

We screened several mutants from mutagenized *FIL*p::*GFP* transformants, which express GFP on the abaxial side. Using stereoscopic microscope, we obtained two different types of mutants: 18 T2 plants, in which GFP was detected on the upper surface (adaxial side) of the young rosette leaves, and 256 T2 plants, in which the GFP fluorescence was weak or not detected. Furthermore, we analyze the former type of the mutants using CLSM to confirm in which region GFP was expressed in cellular level. We acquired 11 lines that expressed GFP in wider region than WT. Several lines of these mutants had aberrant leaves. We analyze one of them, *#1-63*. This mutant shows the variation of shapes of the leaves: narrow leaves, variegated leaves, and filamentous leaves. In this mutant, some leaves expressed GFP in broader region, but other leaves expressed GFP in narrower region. We will also report the expression pattern of some adaxial-side-specific markers in the mutant and cloning of a responsible gene is still in progress.

220 *NO VEIN*, a Gene Necessary for Leaf-Vein Formation in *Arabidopsis*

Ryuji Tsugeki, Yoshinori Sumi, Kiyotaka Okada

Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Japan

Toward understanding the mechanisms of formation of leaf-venation pattern, we have isolated and analyzed *Arabidopsis* mutants defective in formation of leaf veins. Rosette leaves of *no vein-1* mutant (*nov-1*) are narrow and have much fewer veins than those of wild type. No vein is often observed in the first two rosette leaves of *nov-1*. Expression patterns of *ATHB8* in *nov-1* suggest that *NO VEIN* gene (*NOV*) is necessary for prepro/procambial cell formation in leaves. Local distribution of auxin, which is dynamically changed during the leaf development, is known to be prerequisite for leaf-vascular formation. In *nov-1*, *DR5* expression is disrupted, often confined to the margin in leaves. As judged by *DR5* expression in response to 2,4-D, auxin response in *nov-1* is comparable to that in wild type. These data suggest that *NOV* is required for proper auxin distribution during the leaf development. *nov-2~5* mutants, which are likely to be null alleles, exhibit embryo-defective phenotype such as reduction and fusion of cotyledons and abnormality in the early step of vascular development. It has been reported that similar phenotype was observed in mutants defective in polar transport of auxin. In the developing embryos of the null *nov* mutants, *DR5* is ectopically expressed in the tips of cotyledon primordia and in the suspensor, suggesting that *NOV* is required for proper auxin distribution also in the embryonic development. Taken together, these suggest that *NOV* is required for formation of developmentally regulated auxin-distribution pattern both in the leaf and in the embryo, and thereby necessary for development of the leaf vein and embryo. *NOV* encodes a novel protein. The phenotype of *nov* mutants and expression pattern of marker genes in *nov* mutants will be presented.

221 The distribution of microRNA165/166 in Arabidopsis involves ASYMMETRIC LEAVES2 and histone deacetylase

Yoshihisa Ueno[1], Chiyoko Machida[2], Yasunori Machida[1]
[1]Nagoya University, [2]Chubu University

Tissue-specific expression of microRNAs (miRNAs) plays important roles in the development of multicellular organisms. miRNAs are transcribed as long precursors and are processed by factors that include Dicer-family proteins. However, the regulation of tissue-specific expression of miRNA is poorly understood. Here, we present evidence that the ASYMMETRIC LEAVES2 (AS2), ASYMMETRIC LEAVES1 (AS1), HDT1/HD2A and HDT2/HD2B proteins, all localized to nuclei, control the abaxial (peripheral) distribution of miR165 and/or miR166 (miR165/166) and, consequently, the polarity of Arabidopsis leaves. Following treatment with inhibitors of histone deacetylases (HDACs), as1 and as2 mutants frequently formed abaxialized filamentous leaves. Our reporter gene showed that the distribution of miR165/166 is deregulated in these mutants, especially on the adaxial side of leaves, in addition to the inhibition of HDAC activity. It is known that PHABULOSA (PHB) and REVOLUTA (REV) are negatively regulated by miR165/166 and positively regulate cell identity on the adaxial side of leaves. The recessive REV mutation enhanced the phenotypes of as1 and as2 mutants. The mutation of HASTY (HST), which positively regulates the accumulation of miRNAs, partially suppressed the phenotypes of as1 and as2 mutants. The dominant phb-1D mutation, whose transcripts are resistant to miR165/166, was epistatic to the as2 mutation. Inducible ectopic overproduction of AS2 protein caused decreased accumulation of miR165/166 and ectopic accumulation of PHB transcripts. The reduction of miR165/166 by AS2 required AS1 and HDAC activity. AS2 is expressed on the adaxial side of young leaves. Thus, AS2 together with AS1 and HDAC(s) control the distribution of miR165/166 in leaves of Arabidopsis. Furthermore, we developed RNA interference (RNAi) libraries specific to HDACs and identified HDT1/HD2A and HDT2/HD2B as the relevant factors. HDT1/HD2A and HDT2/HD2B encode the HDACs involved in the regulation of chromatin status. Our findings suggest that these unexpected factors control the specific spatial expression of miRNAs.

222 Addressing the Movement and Possible Target of the *bypass1* Root-Derived Signal

Jaimie Van Norman, Leslie Sieburth
University of Utah

Plants with a mutation in the *BYPASS1 (BPS1)* gene exhibit defects in root development and arrest of apical development. Root excision and grafting experiments revealed that *bps1* (mutant) apical development proceeds normally in the absence of the *bps1* (mutant) root, and the *bps1* root is sufficient to arrest leaf expansion and initiation in the wild type[1]. These data suggest a model where BPS1 negatively regulates production of a root-to-shoot signal that inhibits apical development. BPS1 encodes a novel plant specific protein of unknown function and contains no functionally characterized domains. We have used genetics and inhibitors to characterize the *bps1* root-derived signal; our data indicate *bps1* mutants produce a novel carotenoid-derived signal[2].

To understand more about the production and movement of the *bps1* root-derived signal we are taking genetic and reporter gene approaches. We have replicated our surgical ablation of the *bps1* root genetically, by generating double mutants with *ROOT MERISTEMLESS1 (RML1)*. *rml1* seedlings lack post-embryonic cell division in the root[3], with only modest shoot defects. Our data show *bps1* leaf development is rescued in *bps1* rml1 double mutants, confirming our previous surgical data.

How does the *bps1* signal reach the shoot? We reasoned that movement could occur through the xylem or phloem, or by cell-to-cell movement. To distinguish between these possibilities, we generated double mutants between *bps1* and mutants that are defective either in xylem or phloem development. Our data suggests that *bps1* signal moves via a nonvascular route.

Another of our goals is to identify the molecular targets of the *bps1* signal in the shoot. We previously showed that *bps1* mutant leaves were unable to activate expression of the DR5::GUS reporter gene when we applied exogenous auxin (2,4-D)[1]. We are expanding this analysis to include other auxin reporter genes. The results of these studies will be presented.

1. Van Norman, et al., 2004 Current Biology, 14:1739-1746.
2. Van Norman and Sieburth, 2006, manuscript in preparation.
3. Vernoux, et al., Plant Cell, 12:97-109.

223 Quantitative Trait Analysis of Arabidopsis thaliana Root Behavior on a Tilted Hard Agar Surface

Laura Vaughn, Patrick Masson
University of Wisconsin - Madison

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

224 Connecting Auxin Function & Sugar Metabolism: the FANTASTIC FOUR (FAF) Protein Family in Arabidopsis thaliana Development

Vanessa Wahl, Luise Brand, Sarah Fehr, Markus Schmid
MPI for Developmental Biology

Based on expression profiling of flowering time mutants (1), we chose a previously uncharacterized, plant specific gene family, now called FANTASTIC FOUR (FAF), with four members in Arabidopsis for further investigation.

Expression of the FAF genes is extensively regulated throughout development (2). RNA in situ analysis and reporter lines demonstrate that all four FAF genes are strongly expressed in the vasculature, including the pro-vasculature, but individual FAFs are present in distinct domains that overlap only partially. Constitutive expression of the FAF genes resulted in phenotypic effects that appeared auxin related, including defects in the vasculature. Knock-down of the FAF genes led to distortion of the vein formation in leaves, demonstrating that the FAF genes play a critical role in regulating vascular development.

To gain insight into the function of the FAF gene family during Arabidopsis development we have carried out microarray analysis on plants constitutively expressing individual FAF genes. All lines show a deregulation of auxin regulated genes. In addition, expression of enzymes involved in sugar metabolism, especially trehalose synthesis, and lignin as well as flavonoid biosynthesis were affected as well.

(1) Schmid et al., Development, 2003, 130, 6001.
(2) Schmid et al., Nature Genetics, 2005, 37, 501.

225 WVD2-Like Proteins Regulate Plant Growth Behavior and Overall Morphology

Yan Wang, Jessica Will, Robyn Perrin, Christen Yuen, Patrick Masson
University of Wisconsin-Madison

Arabidopsis plants overexpressing WVD2 (WAVE-DAMPENED2) exhibit dampened root waving and leftward root skewing when grown on tilted agar surfaces, compared with the wild-type seedlings. They also have shorter and stockier organs, and their petioles are twisted left-handedly. There are seven WVD2-like (WDL) genes in the Arabidopsis genome, which share high similarity to WVD2 in a conserved region called the KLEEK domain (Yuen et al., 2003, Plant Physiology 131: 493-506). Using RT-PCR, we show that these WDL genes are expressed in all tissues examined, including cotyledons, hypocotyls, seedling roots, stems, flowers, and rosette and cauline leaves. Analysis of transgenic plants carrying the WDL-GUS transcriptional reporter construct reveals that both WDL1 and WDL4 are highly expressed in young leaves of seedlings, but show complementary expression patterns in roots. Overexpressing lines for most of the WDL genes show phenotypes similar to WVD2-overexpressing lines. T-DNA insertional knockout mutants in WDL4 and WDL5 exhibit stronger rightward skewing on tilted agar plates containing propyzamide or oryzalin (two microtubule-destabilizing compounds) relative to the wild-type Columbia (Col) seedlings. T-DNA insertional mutants in WDL6 or WDL7 show altered root growth sensitivity to these compounds but maintain wild-type root skewing. These results suggest that the WDL genes do not show complete redundancy in regulating root growth and development, and at least some of the WDL genes may encode proteins that regulate microtubule organization and/or dynamics. We are examining the global expression patterns and cellular localization of the WDL proteins using GFP and GUS as reporters.

Supported by grants from NASA, NSF and HATCH. RMP was supported by NIH postdoctoral fellowship.

226 The Angiosperm Stem Cell Niche: Recruitment of the WUS/CLV Feedback Loop for Leaf Development in Grasses

Judith Nardmann, Wolfgang Werr
Institute of Developmental Biology, University of Cologne

In Arabidopsis, stem cell homeostasis in the shoot apical meristem (SAM) is controlled by a feedback loop between WUS and CLV functions. WUS orthologues were identified in maize and rice by detailed phylogenetic analysis of the WOX gene family. The allotetraploid maize genome contains two WUS paralogues (ZmWUS1 and ZmWUS2), whereas a single WUS orthologue is present in the rice genome (OsWUS).

None of the isolated grass WUS orthologues displays an organizing centre-type expression pattern in the vegetative SAM such as in Arabidopsis. In contrast, the maize and rice WUS expression patterns relate to the specification of new leaf phytomers. The WUS patterns are consistent with the transcriptional activity of TD1 and FON1 encoding CLV1 orthologues of maize and rice, respectively. The co-recruitment of WUS and CLV1 genes for leaf development implies co-selection in two grass species without affecting stem cell homeostasis. However, the maize and rice WUS and CLV1 orthologues are co-expressed in all kinds of reproductive meristems where fasciation and supernumerary floral organs occur in td1 or fon1 loss-of-function mutants. In conclusion, the grass patterns raise doubts about the uniqueness of WUS/CLV antagonism in the maintenance of the shoot stem cell niche and its general applicability for plant species.

227 LEAFY and the Evolution of Rosette Flowering in Idahoa

Michael White, Nicole Van Abel, Marek Sliwinski, David Baum
University of Wisconsin

The Brassicaceae species *Idahoa scapigera (Isc)* produces all of its flowers on long pedicels that derive from the axils of rosette leaves (rosette flowering). Unlike most species of Brassicaceae, *Idahoa* has two, divergent *LEAFY (LFY)* paralogs. Previous work showed that *IscLFY1* driven by its native *cis*-regulatory regions could be introduced into an *Arabdiopsis lfy* mutant background and could completely rescue stamen and carpel defects; however, *IscLFY1* transgenic lines were deficient in petal production and showed architectural defects including internode compression and the production of bracteate flowers. Here, we conducted the equivalent experiment with *IscLFY2* and found full rescue of *LFY*-dependent floral traits. *IscLFY2* transgenic lines did not show detectable internode compression or bract production, but a significant proportion of the lines produced terminal flowers. When introduced into an *IscLFY1*;*lfy* genetic background, *IscLFY2* rescued the architectural and petal-loss defects, but also yields many lines with terminal flowers. Examination of the *IscLFY2 cis*-regulatory region reveals greater similarity to the equivalent region of *LFY* than was true for *IscLFY1*, which may explain the more complete rescue of the *lfy* mutation. Fusion of the *IscLFY2* 5' *cis*-regulatory region to a GUS reporter gene yielded relatively normal expression in all floral whorls, but additional expression in the inflorescence meristem (IM), which can explain the production of terminal flowers. This result is similar to that found with the *LFY* homolog from another rosette flower species, *Leavenworthia crassa* (*Lcr*). It is plausible that terminal flower production by *IscLFY2* and *LcrLFY* is indicative of a similar change in *LFY* regulation in these two lineages and opens up the possibility that this regulatory change may have contributed to these parallel origins of rosette flowering. The fact that *IscLFY1* shows only incomplete rescue suggests that this paralog is either only partially functional (perhaps en route to non-functionalization) or that the two *LFY* genes of *Idahoa* have been subfunctionalized, with *IscLFY1* playing a role that is not detectable in an *Arabidopsis* genetic background.

228 Cytoplasmically Localized CRY1 Promotes Blue-light-dependent Cotyledon Expansion in *Arabidopsis*

Guosheng Wu, Tessa Durham, Edgar Spalding
Department of Botany, University of Wisconsin, Madison, Wisconsin

Cryptochromes are blue light receptors that mediate many aspects of plant photomorphogenesis, including inhibition of hypocotyl elongation and promotion of cotyledon expansion in seedlings. Null *cry1* mutants develop long hypocotyls, long petioles, and smaller cotyledon blades compared to wild type when grown in blue light. Studies have shown that CRY1 is located both in the nucleus and the cytoplasm. To compare the contributions to seedling development of cytoplasmic and nuclear CRY1, we generated transgenic *cry1* plants expressing *GFP-CRY1* with a nuclear localization signal (*35S::GFP-NLS-CRY1*) or a nuclear export signal (*35S::GFP-NES-CRY1*) and compared their phenotypes with *35S::GFP-CRY1* plants. Confocal analysis of plants overexpressing *GFP-NLS-CRY1* and *GFP-CRY1* showed a strong concentration of CRY1 in the nucleus. The long hypocotyl and petiole phenotypes of *cry1* seedlings grown in blue light were strongly rescued by these constructs. However, the smaller cotyledon area of *cry1* was not rescued. Confocal analysis of plants expressing *GFP-NES-CRY1* showed that CRY1 was located in the cytoplasm in high blue light. Cotyledon growth in these lines was rescued, being promoted even beyond wild-type size. This indicates that in blue light, cytoplasmic CRY1 promotes cotyledon expansion. The hypocotyl and petiole phenotypes were only partially rescued. Either cytoplasmic CRY1 has some influence on these processes or an undetectable trace amount of nuclear CRY1 was responsible for this partial rescue.

229 Wound-Inducible Upregulation of Trichome Density is Dependent on Jasmonic Acid Signaling

Yuki Yoshida[1], Junji Takabayashi[2]

[1]Graduate School of Science, Kyoto University, [2]Center for Ecological Research, Kyoto University

Spacing of trichomes is important for their functions, one of which is a structural barrier against herbivores. Molecular genetic studies have revealed that a family of small MYB proteins, including TRIPTYCHON (TRY) and CAPRICE (CPC), plays a critical role in lateral-inhibition-based patterning of trichomes. Despite the importance of TRY/CPC, it has been suggested that there are still other unknown pathways to regulate trichome density. We aimed to gain insight into such novel aspects of trichome patterning. We focused on the wound-inducible upregulation of trichome density, which was recently reported by Traw and Bergelson (2003). Plastic change in trichome density is observed also in various plant species other than *Arabidopsis*, and is generally considered to be an induced defense against herbivory.

Here we show that *allene oxide synthase* (*aos*) mutant, defective in jasmonic acid biosynthesis, failed to upregulate their trichome density when wounded. In normal growth condition, *aos* mutant produced trichomes comparable to wild type. *CORONATINE INSENSITIVE1* (*COI1*) encodes an F-box protein essential for jasmonic acid signaling. Like *aos* mutant, *coi1-1* mutant also failed to increase their trichomes when wounded. These results indicated that both biosynthesis and SCF^{COI1}-complex-mediated signaling of jasmonic acid are essential for the regulation of trichome density in response to wounding, though not required for the normal differentiation of trichome cells. In contrast, other mutants with altered response to jasmonic acid, namely *jasmonate insensitive1-7* (*jin1-7*) and *jasmonate resistant1-1* (*jar1-1*), did not show any defects in the wound-responsive upregulation of trichome density, indicating that JIN1 and JAR1 are not required for the jasmonic acid signaling involved in the regulation of trichome density.

Interestingly, *try-29760* and *cpc-2* mutants, defective in the lateral inhibition of trichomes, were able to upregulate their trichome density when treated with jasmonic acid. This raised the possibility that the effect of jasmonic acid on trichome patterning acts independently from TRY/CPC. Currently, we are further investigating the relationship of jasmonic acid signaling with TRY/CPC dependent pathway of epidermal pattern formation.

Traw and Bergelson, Plant Physiology 133: 1367-1375 (2003)

230 Studies of Auxin Inducible Genes in Association with Early Transdifferentiation Process into Tracheary Elements Using cDNA Microarray

Saiko Yoshida[1], Taku Demura[2], Hiroo Fukuda[1]

[1]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, [2]Plant Science Center, RIKEN

Apical-basal polar auxin transport is believed to function in the formation of a continuous vascular strand. Polar auxin transport inhibitors cause ectopic vascular formation at leaf margins and parallel vascular strands at the central regions. We previously founded that an auxin transport inhibitor, NPA prevented tracheary element (TE) differentiation from isolated Zinnia mesophyll cells and auxin overcame its prevention. Furthermore, detailed analysis showed that intracellular free NAA was decreased by the treatment of NPA (Yoshida et al., Plant and Cell Physiology, 46, 2019-28, 2005). It suggested that NPA prevented TE differentiation by decrease of active form of NAA, and the addition of excess amount of NAA may compensate this depletion. In this study, we performed microarray analysis of genes expressed in NPA-treated cells and NPA plus NAA-treated cells, to get an insight into auxin regulations of transdifferentiation. The systematic gene expression analysis revealed that NAA suppressed the expression of wound response genes and promotes the expression of early xylogenesis/procambium formation-related genes. NAA promoted the expression of Arabidopsis gene homologues related to auxin signaling, auxin influx, hormone biosynthesis, hormone metabolism, transcription and transport at early stage of transdifferentiation. Based on these results, auxin action at the transdifferentiation into tracheary elements is discussed.

231 Overexpression of Arabidopsis SOB5 Suggests the Involvement of a Novel Family of Plant Proteins in Cytokinin-mediated Development.

Jingyu Zhang[2], Elizabeth Wrage[2], Radomira Vankova[1], Jiri Malbeck[1], Michael Neff[2]

[1]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR, Rozvojova 135, 165 02 Prague 6, Czech Republic, [2]Department of Biology, Washington University, St. Louis, MO 63130

Activation tagging, a gene-overexpression mutagenesis tool, has been used to identify extragenic suppressors of the long-hypocotyl phenotype conferred by the photoreceptor mutant phyB-4. This mutant screen allows us to cast a broad net in search of potentially redundant genes involved in seedling development. The sob5-D mutation confers phenotypes typical of transgenic plants with elevated levels of the plant hormones, cytokinins. The sob5-D mutation is caused by the overexpression of a novel gene, SOB5, which is part of previously uncharacterized family of plant-specific proteins. A translational fusion between SOB5 and the green fluorescent protein reporter was localized in the cytoplasm as well as associated with the plasma membrane when transiently expressed in onion epidermal cells. Analysis of transgenic plants harboring a SOB5:SOB5-B-glucuronidase (GUS) translational fusion under the control of the SOB5 promoter region showed GUS activity in vegetative tissues (hydathodes and trichomes of leaves, shoot meristems and roots) as well as in floral tissues (pistil tips, developing anthers and sepal vasculature). Cytokinin quantification analysis revealed that adult sob5-D plants accumulated higher levels of trans-zeatin riboside, trans-zeatin riboside monophosphate and isopentenyladenine 9-glucoside when compared to the wild type. Consistent with this result, AtIPT3 and AtIPT7 were found to be up-regulated in a tissue-specific manner in sob5-D. Physiological analysis of sob5-D demonstrated reduced responsiveness to exogenous cytokinin in both root-elongation and callus-formation assays. Though our data suggest a role for a novel gene, SOB5, in cytokinin-mediated plant development, knock-down and knock-out mutants in SOB5 are phenotypically similar to the wild type, suggesting that other SOB-five-like (SOFL) proteins in Arabidopsis may be functionally redundant with SOB5. In support of this hypothesis, overexpression of AtSOFL1 and AtSOFL2 confers phenotypes similar to sob5-D. We are further testing this hypothesis by generating double and triple knock-down and knock-out mutants between SOB5, AtSOFL1 and AtSOFL2.

232 Antisense-expression of the OSCP(δ) Subunit of Mitochondrial ATP Synthase Promotes Mitochondrial Division in Arabidopsis

Mary Robison, Matthew Smid, Xingyuan Ling, David Wolyn

Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada, N1G 2W1

The mitochondrial and chloroplastic ATP synthases of plants are homologous multi-subunit complexes consisting of intrinsic F_0 and extrinsic F_1 segments connected by a stator stalk. The oligomycin sensitivity-conferring protein (OSCP) subunit which forms part of the stator stalk in mitochondria is homologous to the δ subunit in chloroplasts. To specifically reduce mitochondrial ATP synthesis, we transformed Arabidopsis with an antisense copy of the mitochondrial OSCP (AF380647) under the control of a dexamethasone-inducible promoter. Seed homozygous for the transgene, imbibed on dexamethasone-containing media in the light, died shortly after germination, but imbibition in the dark resulted in etiolated seedlings of short stature. Treatment of established soil- or hydroponically-grown plants with dexamethasone resulted in slow growth and development, strap-like or cupped leaves that often had wavy margins, slight chlorosis, and cup-like sepals that restricted normal pollination. The magnitude of response was dose-dependent. Crossing the anti-OSCP plants with a line expressing GFP targeted to the mitochondrion (Logan and Leaver, 2000) allowed us to examine effects on mitochondrial morphology. Dexamethasone-treated plants had increased numbers of small mitochondria, usually arranged in chains. Northern analysis of genes related to mitochondrial fission or fusion demonstrated up-regulation of several fission-related DRP genes after dexamethasone treatment whereas the fusion-related genes were unaffected.

X. Ling, current address:Department of Botany, PO Box 3165, University of Wyoming, Laramie WY 82071-3165

233 Photoperiod-Sensitive Phase for Time to Flowering in *Arabidopsis thaliana* Landsberg *erecta* as Revealed by Reciprocal Transfer Experiments

Daniel Villegas, Jose Alcalde

Facultad de Agronomia e Ingenieria Forestal. Pontificia Universidad Catolica de Chile. Casilla 306-22 Santiago. Chile.

Photoperiod (P) and temperature (T) are the most important environmental factors regulating time to flowering in most annual and biennial plants. Among photoperiod sensitive plants some are induced by a single inductive day/night cycle (e.g. *Sinapis alba*, *Lolium temulentum*, *Pharbitis nil*), and may not flower if they are not exposed to sufficiently long or short photoperiods to induce flowering, depending on whether they are long or short day plants, respectively. Others have a long photoperiod-sensitive phase, requiring several photoperiodic cycles to become induced. Ellis *et al.* [Ann. Bot. 70: 87-92, 1992] have proposed an analytical procedure to estimate the durations of P-sensitive and P-insensitive phases of preflowering development in annual crop species (e.g. soybean, barley, lentil, maize) by reciprocally transferring plants grown in long days (LD) to short days (SD) and vice versa at different times after sowing. By analyzing the durations from sowing to flowering (*f*) of the whole dataset the relative durations of preflowering subphases are estimated.

The application of this approach to *Arabidopsis thaliana* Landsberg *erecta* indicated that: a) Plants grown under 10 h d^{-1} photoperiod (SD) flowered 32 days later than plants grown under 20 h d^{-1}, at a mean 21.0 °C temperature. b) A photoperiod-sensitive phase for time to flowering was detected, starting approximately at 10 days after sowing and lasting for 35 days under either LD or SD. c) Plants transferred from LD to SD and vice versa at different times during this sensitive phase modified their flowering times gradually, implying a quantitative modulation of time to flowering by photoperiod. d) A post sensitive phase of variable length depending on photoperiod perceived during the previous sensitive phase accounted for the remaining time to flowering (corolla color visible). This behavior is different from what was expected from plants tested previously in which differences in flowering time were explained by differences in the duration of the photoperiod sensitive phase, and not in the duration of the post-sensitive phase. Discussion is centered on whether described molecular signaling systems are compatible with results obtained, and on whether these results could be paralleled by expression of candidate genes modulating time to flowering in Arabidopsis.

Researched funded by FONDECYT, Chile. Project No 1040551. A CONICYT Scholarship for doctoral studies for D.V. is gratefully acknowledged.

234 Whole Gene Family Expression and Drought Stress Regulation of Aquaporins

Erik Alexandersson, Vamsi Moparthi, Urban Johanson, Per Kjellbom

Department of Biochemistry, Lund University, Lund, Sweden

In *Arabidopsis thaliana* aquaporins form a large family of proteins with 35 members. These can be divided into four subfamilies: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin 26-like proteins (NIPs) and small basic intrinsic proteins (SIPs). PIPs and TIPs have been shown to act as water channels, even though some isoforms have also been shown to transport small solutes such as urea and ammonia. NIPs are believed to facilitate glycerol transport in plants, whereas the substrate specificity for SIPs still is unknown. Since many aquaporins act as water channels, they are thought to play an important role in plant water relations. It is thus of interest to study individual expression patterns of aquaporin isoforms in order to further elucidate their involvement in plant water transport.

Earlier, we monitored the expression patterns of all 35 Arabidopsis aquaporins in leaves, roots and flowers by cDNA microarrays, specifically designed to avoid cross-reaction of highly homologous aquaporin isoforms, and by quantitative real-time reverse transcriptase PCR (Q-RT-PCR)[1]. In this way we could show that many aquaporins are pre-dominantly expressed in root or flower organs, while none seem to be leaf specific. Looking at the subfamilies, most PIPs and some TIPs have a high level of expression, while NIPs are present at a much lower level. Upon gradual drought stress, we showed that PIP transcripts are generally down-regulated in leaves, with the exception of *AtPIP1;4* and *AtPIP2;5*, which are up-regulated. *AtPIP2;6* and *AtSIP1;1* are constitutively expressed throughout the drought stress.

In order to further study the PIP isoforms displaying different regulation during drought stress, we fused the promoters of AtPIP1;4, AtPIP2;5 and AtPIP2;6 with a reporter gene (GUS) and were in this way able to establish distinct expression patterns for the three gene transcripts. We will also localise AtPIP1;4 and AtPIP2;5 on the sub-cellular level by GFP-fusions. Furthermore, we could demonstrate that the PIP isoforms show the same kind of pattern of up- and down-regulation during drought stress in five Arabidopsis ecotypes with different water use efficiency.

1) Alexandersson et al. 2005, Plant Mol Biol 59:469-484.

235 Ethylene Signaling Components Are Involved in Polycyclic Aromatic Hydrocarbon Stress Responses in *Arabidopsis thaliana*

Adan Colon-Carmona, Merianne Alkio, David Weisman
University of Massachusetts-Boston

With the growing awareness of hazardous polycyclic aromatic hydrocarbons (PAHs) to humans, the interest in plant responses to these environmental pollutants is increasing. We studied ethylene signaling in plant PAH stress by 1) analyzing stress responses of Arabidopsis mutants, defective in different components of the ethylene-signaling pathway, to the model PAH, phenanthrene; 2) studying ethylene-inducible gene expression in PAH-exposed GUS-reporter plants and 3) comparing global gene expression changes of PAH- and ethylene-exposed plants. Results: 1) PAH-exposed wild type plants (wt) had shorter hypocotyls and roots than wt grown on control medium. However, growth of some ethylene insensitive mutants (*etr1-1*, *ers2-1*, *ein2*, *ein6*) was even more inhibited by PAH than that of wt, whereas constitutive ethylene signaling mutants (*eto3*, *ctr1-1*, and the triple mutants *etr1-6xetr2-3xein4-4* and *etr2-3xers2-3xein4-4*) generally appeared resistant to the inhibition of organ elongation, and often even had longer roots on PAH than on control medium. The mutants did not differ from wt in respect to PAH-induced oxidative stress. However, compared to wt, the rosettes *ctr1-1* and *etr1-7* were more reduced in size and more chlorotic, indicating greater PAH sensitivity. 2) After PAH-exposure, GUS expression was slightly induced in *chitinase-GUS* plants but strongly induced in *GSTF2-GUS* plants. 3) DNA microarray analyses (our own, and published data) support the view that parts of the ethylene signaling pathway are involved in the PAH response, although ethylene signaling pathway as a whole is not switched on. We conclude that while PAH stress signaling in plants overlaps with other signaling pathways, it involves a unique subset of the known molecular players as well as unidentified cellular components.

Funded by NSF-IBN 0343856

236 The Magnitude of Phosphate-Starvation Responses is Determined by the Rate of Plant Growth and Cell Division

Fan Lai, Peter Doerner
Edinburgh University

Plants require adequate and balanced quantities of mineral nutrients for optimal growth, but such conditions are rarely found in nature. For example phosphate (Pi) is required for RNA and DNA synthesis, phospho-lipids, in intermediate energy metabolism and to regulate protein function. We examined the relationship between Pi starvation responses and plant growth. To assess Pi starvation responses, we quantified gene expression responses of a set of Pi-responsive genes. When plant growth was globally enhanced, e.g. by supply of sugars, Pi starvation responses were enhanced. We examined whether this was a nutrient-specific effect or caused by altered growth magnitude. When plant growth was selectively inhibited (e.g by osmotic stress) starvation responses were significantly reduced. Selective growth inhibition (for example of root growth by elevated nitrogen supply) led to reduced Pi starvation responses in roots. These data show that the magnitude of Pi starvation responses is determined by the demand for phosphate. We found that the magnitude of cell proliferation, not cell expansion was responsible for setting phosphate demand. Moreover, starvation responses appear to be controlled organ autonomously. This is in contrast to previous suggestions that plant phosphate status is sensed in the shoot (Burleigh and Harrison 1999). We propose a model in which growth control networks regulating meristem activity and therefore shoot-root mass ratios, which then sets the level of demand for phosphate in plant organs. Altered allocation of growth potential, e.g. by altering carbon-nitrogen ratios, is independent of phosphate nutritional status and dominates over phosphate starvation-induced growth responses.

Burleigh, S. H. and M. J. Harrison (1999). Plant Physiology 119: 241-248.

237 Chilling Stress is Associated with ROS Overload in Roots and Leaves

John Einset
Norwegian University of Life Sciences

Although normally considered as chilling-tolerant, Arabidopsis plants exposed to 2 days of chilling at 4 C show elevated levels of reactive oxygen species (ROS) and inhibited root growth for up to 4 days when transferred back to optimal growing conditions. During this recovery period, ROS levels decline in root tips and in leaves. If plants are pretreated with glycine betaine (GB) prior to the chilling treatment, ROS levels do not increase during chilling and optimal growth begins as soon as plants are transferred back to normal growing conditions without a recovery period. Using microarray technologies, we can show that GB up-regulates several genes in both roots and leaves that reinforce intracellular processes protecting cells from oxidative damage and others that appear to be involved in setting up a scavenging system for reactive oxygen species in cell walls. In roots, GB-activates genes for transcription factors, membrane trafficking proteins (RabA4c, RabB1b), cell wall peroxidases (ATP3a, ATP15a), superoxide dismutases in the cytoplasm, plastids and mitochondria, a mitochondrial catalase, the root specific NADPH-dependent ferric reductase (FRO2) localized to the plasma membrane as well as glutathione and ascorbate metabolizing enzymes in the cytoplasm and cell wall. Genes activated in leaves include transcription factors, several intracellular ROS metabolism enzymes as well as membrane trafficking components. In addition, specific extracellular peroxidases are activated by GB in leaves as well as a plasmamembrane NADPH-dependent ferric reductase (FRO6). Experiments with knockout mutants provide direct evidence that two of the GB-activated genes, one gene coding for a membrane trafficking protein (RabA4c) and the other coding for a putative bZIP transcription factor, are required for GB's effects on recovery from chilling and ROS accumulation during chilling. GB does not prevent chilling stress in these knockouts. Experiments with RabA4c promoter-GUS and -YFP transgenics show that RabA4c expression coincides with regions of most active ROS accumulation in vascular tissues during chilling stress. Taken together, these results plus the fact that application of ROS directly to plants in the absence of chilling can cause root growth inhibitions suggest that ROS may be the cause of chilling stress.

238 An Arabidopsis thaliana MAPK (At3g45640/AtMAPK3) is involved in glucose and ABA responses

Jaime Aportela Cortez, Patricia Leon Mejia, Angel Arturo Guevara-Garcia
Instituto de Biotecnologia, UNAM Cuernavaca, Mor., Mexico

Mitogen activated protein kinases (MAPKs) signaling cascades are involved in the control of abiotic and biotic stress responses in all organisms. Glucose plays an important role as carbon source for plant growth and development, but also as signal to regulate metabolism, differentiation and stress responses. Abscisic acid (ABA) participates in the control of seed germination and mediates plant responses to some kind of abiotic stress. The co-regulation of several plant genes by both glucose and ABA has been reported, but although the cross-talk between this two signal transduction pathways is experimentally supported, little is known about the molecular basis of their interaction. Previously has been reported that compared with wild-type plants, Arabidopsis thaliana transgenic lines overexpressed a stress-activated MAPK (At3g45640/AtMPK3) were more sensitive to ABA-triggered postgermination growth arrest (1). Here, we compare the performance of A. thaliana transgenic lines with high (35ScaMV-AtMPK3), and low (RNAi/AtMPK3) levels of expression of the AtMPK3 gene. Our results show than ABA-hypersensitive AtMPK3 overexpressed lines are also glucose-tolerant, whereas AtMPK3 suppressed lines are lightly ABA-tolerant and apparently glucose-sensitive. Those findings implicate that both ABA and glucose signaling pathways, seems to converge in a stress-activated MAPK cascade, in which AtMPK3 is involved. Our studio includes: a) the phenotypic characterization in different developmental stages of transgenic lines growing in ABA and glucose; b) an analysis of expression of the AtMAPK3 gene in response to ABA and glucose on wild-type plants; and c) an analysis of expression of some ABA/glucose responsive genes, on wild-type and transgenic plants, treated with ABA and glucose.

1) Lu, C., Han, M-H., Guevara-Garcia, A. and Fedoroff, N. (2002). Mitogen-activated protein kinase signaling in post-germination arrest of development by abscisic acid. PNAS, USA. , 99 (24):15812-15817.

239 Accumulation of Reactive Oxygen Species in Ovules Reduces Plant Fertility

Yahya Mohammed, D. Head, Bernard Hauser
University of Florida, Gainesville, FL 32611-8526

In response to environmental stress in many plants, ovules and seeds degenerate during their development, dramatically reducing the number of seeds and fruits produced. In Arabidopsis, salt stress induces programmed cell death (PCD) in ovules, but not in the surrounding carpel walls and the transmitting tract. Transcript levels from healthy ovules were compared with those found in ovules when they commit to undergo PCD. The expression of transcripts encoding some enzymes that detoxify reactive oxygen species (ROS) decrease once ovules committed to abort. These changes in gene expression coincided with the accumulation of ROS in female gametophytes. Because ROS activate genetic programs that trigger PCD, the rates of ovule abortion and fertility were examined in ROS scavenging mutants. Of the loci that exhibited significant changes in gene expression following salt stress, two peroxidase mutants and a superoxide dismutase (SOD) mutant were evaluated. When compared to wild-type plants, the *sod* mutant accumulated ROS in healthy ovules. Qualitative assays reveal that both peroxidase mutants have elevated ROS levels in healthy and stressed ovules. In these peroxidase mutants, increased accumulation of ROS led to moderate, but significant, decreases in plant fertility. This result indicates that ROS accumulation is sufficient to reduce fertility. Interestingly, reduced ROS scavenging attenuated fertility, but did not eliminate it. This indicates that multiple control points adjust reproduction to match environmental conditions.

240 An Arabidopsis Cyclin –Like Protein, CLP, Is Involved in Drought Stress Response and Plant Development

Yin Hua Jin[1], Jing Bo Jin[1], Dae-Jin Yun[2], Paul Hasegawa[1], Ray Bressan[1]
[1]Purdue University, [2]Gyeongsang National University

The *Arabidopsis* genome contains 50 putative cyclin proteins, But only very few functional studies of plant cyclins have been reported to date. Here we show that mutation of cyclin-like protein, CLP, cause drought sensitive phenotype and abnormal plant organ development. The clp mutant plant was originally obtained from screening of Arabidopsis T-DNA insertion mutant pools in the C24 background, which carry the firefly luciferase reporter gene driven by RD29A promoter (RD29A::LUC). The clp mutant plant showed extremely high constitutive bio-luminescence level, without any treatment. Genetic analysis of F1 and F2 backcrossed plants revealed that the mutation is dominant and mutant phenotype is caused by single mutation. The *clp* plant exhibit extreme drought sensitive phenotype and the phenotype could not be rescued by exogenous ABA treatment. Moreover, the *clp* plant shows greater water loss compare to wild-type plant. The *clp* seedling development is insensitive to ABA . The *clp* plants also exhibit wider rosette leaves and increased numbers of trichomes on the rosette leaves and stems compared to C24 wild type plants. The *clp* plants also have abnormal petal and stamen numbers in the terminal flowers. These results suggest that the CLP plays important roles in ABA response and maintenance of plant organ morphology.

241 rcd3 mutation disrupts abscisic acid, reactive oxygen species and nitric oxide induced stomatal closure in Arabidopsis mutation disrupts abscisic acid, reactive oxygen species and nitric oxide induced stomatal closure in Arabidopsis

Triin Kollist
University of Tartu

Ozone (O_3), the predominant air pollutant, has been shown to be a useful tool to induce acute formation of reactive oxygen species (ROS) and to identify molecular components regulating ROS induced processes in leaf cells. We have previously identified a series of O3-sensitive *rcd* (for *r*adical induced *c*ell *d*eath) mutants. Here we describe *rcd3* which phenotype is related with stomatal function. *rcd3* has constitutively higher stomatal conductance than the Col-0 wild type. Also O3-induced stomatal closure is largely suppressed in *rcd3.* The number of stomata in the abaxial side of the leaf is not altered. Detailed analysis of stomatal responses right after the onset of O3-treatment revealed that O3 induces a rapid stomatal closure and subsequent reopening within 30 minutes in most of the mutants and ecotypes including Col-0 and L*er*. Interestingly, in the abscisic acid (ABA) insensitive mutant's *abi1, abi2, ost1* and in *rcd3,* the transient closure was absent. The phenotype of *rcd3* is not caused by altered ABA content since a six hour treatment with O3 caused similar ABA induction in both Col-0 and *rcd3*. However, guard cells of *rcd3* are insensitive to ABA, hydrogen peroxide and nitric oxide treatment measured as reduction of stomatal aperture. It is likely that map-based cloning of *rcd3*, currently underway, will reveal a new regulator for the signal transduction pathway leading to stomatal closure.

242 Reciprocal leaf and root response to nitrogen stress in Arabidopsis thaliana

Cawas Engineer, Robert Kranz
Washington University in St. Louis

Nitrogen is an essential macronutrient for plant growth and survival. We analyzed the temporal and spatial onset of nitrogen stress sensing in Arabidopsis using the promoter of a high affinity ammonium transporter, AtAmt1.1. An AtAmt1.1-Gal4 driver line with three 5XUAS reporters (LUC, GFP, and GUS) facilitated the in vivo profiling of the whole plant and individual cells throughout the plant. The gene is expressed in the roots only under nitrogen stress and only in the leaves under nitrogen sufficient conditions. This thirty-five fold reciprocal expression indicates that Arabidopsis undergoes rapid resource reallocation in plants grown under different nitrogen supply regimens. Ultimately, nitrogen stress-mediated reallocation results in root architectural restructuring. The GFP and GUS reporters facilitated the temporal and spatial analyses within the cell types of root and aerial tissues. We propose a model for the precise timing of this nitrogen stress response from the perspective of AtAmt1.1-expression profiling.

243 Intracellular Production Of Reactive Oxygen Species During Salt Stress Is Mediated By PtdIns-3-Kinase Regulated Endocytosis

Yehoram Leshem

Department of Plant and environmental Sciences, Institute of Life Sciences

Oxidative stress is a common response of plants to most of abiotic stresses. Here we show that during salt stress ROS are produced in Arabidopsis root tips within minutes in response to the ionic but not the osmotic stress. ROS production was significantly reduced in Atrboh (phox) mutants as well as by DPI, indicating NADPH Oxidase (Phox) mechanism of ROS production. Confocal microscopy study showed that the ROS were detected in cytosolic speckles which were suspected to be mitochondria but were found to be endosomes as detected by specific endosomal membrane dyes. These findings suggested the involvement of endocytosis in induction of the NADPH oxidase. Moreover Z stack analysis of a single cell showed that these endosomes were not found near the plasma membrane but rather integrated within the tonoplast suggesting the involvement of vacuolar transport of the ROS signal. Both the confocal microscope findings and the Atrboh mutants results suggested that intracellular vesicle trafficking regulated the induction and the transduction of the ROS during salt stress. Indeed treatment of WT seedlings with vesicle trafficking inhibitors such as LY and Brefeldin A abolished completely the induction of ROS and endocytosis during salt stress. Recent studies in mammalians systems showed that initiation of vesicle budding is induced by phosphorylation of the D3' position of the phosphatidylinositol ring by the PtdIns-3-Kinase. Inhibition of the PtdIns-3 kinase in Arabidopsis seedlings with the fungal inhibitor wortmannin suppressed the endocytosis and reduced ROS production during salt stress. Moreover, similar effects were detected in Arabidopsis mutants in the PtdIns-3 kinase. These processes were restored by supplementation with exogenous PtdInsPs that contained phosphorylated D3' but not D5'. In optimal conditions the mutants looked like wild type but after transfer to high salt they exhibited a salt-overly-sensitive phenotype. Similar results were obtained in wild type plants treated with wortmannin or DPI, underscoring the positive signaling role of ROS. Taken together, these results suggest that vesicle trafficking regulates the production localization and transmission of ROS which in turn regulates the signal transduction during salt stress.

244 An Arabidopsis Subtilase Likely Functions In Salt Stress Responses Through A Mechanism Involving Regulated Intramembrane Proteolysis (RIP)

Jianxiang Liu, Ping Che, Renu Srivastava, Stephen Howell

Plant Sciences Institute, Iowa State University, Ames IA 50011

Arabidopsis encodes over 50 subtilisin-like serine proteases (Rautengarten et al., 2005) and one of them, AtS1P (At5g19660), is similar to mammalian site-1 proteases (S1Ps) which function in ER stress responses and cholesterol homeostasis through a mechanism called regulated intramembrane proteolysis (RIP). Loss-of-function mutations in AtS1P result in heightened sensitivity to salt stress and reduced level of expression of salt stress-induced genes, such as ATHB-7, a homeodomain transcription factor. Three Arabidopsis b-ZIP transcription factors, AtbZIP17, -28 and -49, have structural characteristics of S1P targets in ER stress responses in that they are predicted to be type II membrane proteins with canonical S1P cleavage sites on the luminal side of the membrane. T-DNA insertion mutation in AtbZIP17, blocks the expression of ATHB-7 in response to salt stress and also confers highly sensitivity to salt stress. The results are consistent with a transcription factor signaling cascade in which AtS1P activates membrane associated b-ZIP transcription factors, such as AtbZIP17, which in turn upregulate the expression of salt stress genes, such as ATHB-7. Experiments showing the direct cleavage of transcription factor AtbZIP17 by AtS1P are underway. This system represents a salt stress response and signaling pathway that has not been previously described in plants.

245 How Imidazolinone Kills Plant – The Transcriptome Profiling Of *csr1-2* Upon Imidazolinone Herbicide Treatment

Yuzuki Manabe, Brian Miki

Bioproducts and Bioprocesses, Research Branch, Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada

An herbicide tolerant mutant, *csr1-2*, carries an equivalent mutation to commercially available Clearfield © crops. *CSR1* encodes a catalytic subunit of acetohydroxyacid synthase (AHAS, EC 2.2.1.6), which catalyzes the first step of branched-chain amino acids, Ile, Leu, and Val, biosynthesis. *csr1-2* is a dominant mutation which retains the activity of AHAS but make AHAS resistant towards Imidazolinone due to reduced binding capacities. Despite their widespread usage, the mechanism by which Clearfield © crops gain Imidazolinone herbicide tolerance has not yet been fully characterized.

Transcription profiling combined with physiological characterization of imidazolinone-tolerant mutants will provide further insights into its well characterized biochemical and genetic features. In wild-type plants, primary root growth is inhibited within several hours of Imidazolinone treatment, but inhibition of shoot growth takes 3 days. Both grafting and microarray expression experiment indicate that there is no suppressing signal transduced from the shoot to the roots to inhibit root growth following Imidazolinone treatment. Based on these data, it has been hypothesized that Imidazolinone acts by separate and independent mechanisms upon root and shoot growth. Our data show that Imidazolinone inhibition of the root growth is not due to the death of the root apical meristem, and is reversible. Use of the ATH1 genechip microarray has also revealed that branched-chain amino acid biosynthesis, amino acid transport and senescence take part in the Imidazolinone response.

246 Role of Arabidopsis *Zeaxanthin Epoxidase* Gene in Responses to Osmotic and Oxidative Stresses

Hye-Yeon Seok[1], Hee-Yeon Park[1], Sun-Ho Kim[1], Bo-Kyung Park[1], Ho-Seung Kim[1], Chin Bum Lee[2], Choon-Hwan Lee[1], Yong-Hwan Moon[1]

[1]Department of Molecular Biology, Pusan National University, Busan 609-735, Korea, [2]Department of Biology, Dong-eui University, Busan 614-714, Korea

Stoichiometric conversions of three xanthophylls pigments, involving the cycling between violaxanthin, antheraxanthin and zeaxanthin, are known as xanthophyllcycle. Zeaxanthin epoxidase (ZEP) catalyzes the conversion of zeaxanthin to violaxanthin and antheraxanthin. This step is considered as the first committed step in abscisic acid (ABA) biosynthesis pathway. ABA plays important roles in environmental stress responses and many cellular processes including seed development, dormancy germination and vegetative growth. As an aspect of the xanthophyll cycle, ZEP also is related to the protection of plants from photooxidation because zeaxanthin may protect from light stress by directly quenching free radicals and by making the thylakoidmembrane less permeable to oxygen.

In this study, to elucidate the function of ZEP in stress-response, we have generated transgenic plants overexpressing Arabidopsis *ZEP* gene, and investigated responses to salt-, drought- and oxidative-stresses in the transgenic plants. First, the *ZEP* gene was ectopically expressed under *CaMV35S* promoter in transgenic plants, and the ectopic expression of the *ZEP* gene in the transgenic plants was confirmed by semi-quantitative RT-PCR. The transgenic plants also had almost no zeaxanthin content, indicating the products of the overexpressed ZEP function. Using selected transgenic plants, we had investigated responses to a few salts such as NaCl, LiCl, and KCl with various concentrations. As results, the transgenic plants were more tolerant to Na^+ and Li^+ than wild-type (WT) plants, although responses of the transgenic plants were different between Na^+ and Li^+. The transgenic plants also showed tolerance to mannitol, suggesting that the transgenic plants are tolerant to drought, and indeed the transgenic plants on soil showed tolerance to drought. Interestingly, the transgenic plants were less tolerant to MV than WT, revealing that the transgenicplants are sensitive to ROS.

Taken together, our results suggest that the *ZEP* gene would be very useful to generate transgenic plants tolerant to salt- and drought-stresses.

247 Large-Scale Screen and Isolation of Genes Induced by Photooxidation and Photoinhibition Stresses in Rice

Ji-Sung Han[1], Sun-Ho Kim[1], Dong-Hyouk Woo[1], Ja-Myoung Lee[1], Byoung Yong Moon[2], Gynheung An[3], Choon-Hwan Lee[1], Yong-Hwan Moon[1]

[1]Department of Molecular Biology, Pusan National University, Busan 609-735, Korea, [2]School of Biotechnology and Biomedical Science, Inje University, Gimhae 621-749, Korea, [3]Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang 790-784, Korea

Light is one of the most important environmental factors that control the growth and development of plants. But excess light is harmful to plants and causes the photooxidation of pigments and the photoinhibition of photosynthesis. In this study, to isolate genes induced by photooxidation and photoinhibtion stresses, we have performed two experiments that involve a new reverse transcription-polymerase chain reaction (RT-PCR) using annealing control primers (ACPs) and analysis of promoter activity in promoter trap lines.

First of all, we have isolated 37 differentially expressed genes (DEGs) under photooxidation and photoinhibition stresses using 20 ACPs. Among the 37 DEGs, 22 DEGs showing strong up-regulation (16 DEGs) and down-regulation (6 DEGs) under both stresses were selected for sequence analysis. Basic Local Alignment Search Tool (BLAST) searches revealed that the 22 DEGs represented 21 different genes including S-adenosylmethione synthetase, early nodulin, temperature stress-induced lipocalin, and etc. On the basis of expected functions of the 21 genes, we selected 14 genes for further study. To confirm the results of ACPs, semi-quantitative RT-PCR was performed, and so far 5 genes showed the same expression patterns as in the ACPs results. On the other hand, we analyzed promoter trap lines including pGA2717, a promter trap vector with *GFP*, to isolate promoter induced by highlight and low oxgen. Out of 3500 lines, we selected 103 lines using bioinformatical knowledge that *GFP* is inserted within exon or intron of genes and the orientation of *GFP* is the same as that of gene. We analyzed GFP activity in the 103 lines under highlight and low oxygen conditions in large scale. As results of the analysis, 13 and 5 lines showed the increase of GFP activity under highlight and low oxygen conditions, respectively, and 5 lines showed the increase of GFP activity under both conditions.

Now, we are investigating the biological functions of selected genes especially under photooxidation and photoinhibition conditions.

248 Function of Coactivator Proteins ADA2 and GCN5 in Cold Acclimation in Arabidopsis

Kanchan Pavangadkar[1,2], Nathan Lord[2], Michael Thomashow[1,3,4], Steven Triezenberg[1,2]

[1]Genetics Program, Michigan State University, East Lansing, MI 48824, [2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, [3]DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, [4]Department of Crop and Soil Science

Covalent modifications of histones play important roles in the regulation of transcription. Acetylation of lysine residues on the amino-terminal tails of histones is associated with transcriptionally active genes and is catalyzed by histone acetyltransferases (HAT). GCN5 (a HAT) and ADA2 are components of coactivator complexes such as SAGA in yeast. The coactivator protein ADA2 is essential for the HAT activity of GCN5 in yeast. The *Arabidopsis* genome encodes one homologue of GCN5 and two homologues of ADA2 (ADA2a and ADA2b). Null mutants of *GCN5* and *ADA2b* have pleiotropic effects on plant growth and development whereas *ada2a* mutants show no aberrant phenotype.

Arabidopsis ADA2 and GCN5 physically interact with the transcriptional activator CBF1 which activates the expression of cold-regulated (*COR*) genes during cold acclimation. Cold acclimation is the process by which plants increase freezing tolerance upon exposure to low non-freezing temperatures. CBF1 binds to the cold/dehydration responsive element (CRT/DRE) present in *COR* gene promoters. *ada2b* and *gcn5* mutants show a delay in activation and a reduction in expression of *COR* genes during cold acclimation. Chromatin immunoprecipitation assays show that the acetylation of histone H3 at the *COR* promoters increases upon cold acclimation. ADA2b and GCN5 may also be essential in maintaining the basal expression levels of *COR* genes. We hypothesize that these proteins 'poise' the promoter of *COR* genes in the uninduced state and thus potentiate stronger activation upon induction.

The *Arabidopsis* genome lacks obvious homologs of several SAGA subunits, and thus plant coactivator complexes are likely to be distinct from human or yeast complexes studied previously. To identify components of GCN5-containing coactivator complex(es) in plants, a tandem affinity purification (TAP) cassette encoding a calmodulin binding peptide and IgG binding domain was fused to the N- or C- termini of AtGCN5. The fusion genes were transformed into an *Arabidopsis gcn5* mutant. Lines in which TAP-GCN5 complements the mutant phenotype were used for affinity purification. Western blot analysis of purified fractions suggests that the transcriptional coactivators ADA2a and ADA2b copurify with TAP-GCN5. This study provides a foundation for understanding the biological role and biochemical mechanism of GCN5 in *Arabidopsis*.

249 Application of ABC transporters to enhanced arsenic detoxification

Melissa Pischke, Richard Meagher
Genetics Department, University of Georgia, Athens, GA 30602

The World Health Organization (WHO) recognizes arsenic as a carcinogen and a serious threat to millions of people. While arsenic occurs naturally in the earth's crust, both natural and anthropogenic activities have contributed to arsenic mobilization and increased concentration in the environment, such that WHO guidelines for inorganic arsenic levels are exceeded at many locations, worldwide. One mechanism cells use to deal with exposure to toxicants such as arsenic, is to pump them out of the cytoplasm using a special type of ABC transporter called a multidrug resistance protein (MRP). The yeast MRP, YCF1, actively pumps glutathione:arsenite conjugates into the vacuole, resulting in arsenic resistance. A YCF1 deletion strain lacks pump activity and is hypersensitive to arsenic. We propose that enhanced versions of the YCF1 protein will confer increased arsenic uptake, accumulation, and resistance upon transgenic cells, and the ycf1 deletion strain will provide a convenient experimental system to test this hypothesis. Our short-term goal is identification of evolutionarily conserved residues and domains within MRPs that regulate arsenic toxicity. Our long-term goal is environmental arsenic remediation, leading to a worldwide decrease in arsenicosis. Progress toward the following specific aims will be discussed. (1) Enhance the arsenic-transporting pump activity of YCF1 through PCR mutagenesis. (2) Explore the effects of corresponding mutations on human and Arabidopsis YCF1 homologs. (3) Demonstrate a mutant MRP-mediated increase in plant arsenic resistance, sequestration, and accumulation. Our work will identify MRPs with improved arsenic pump activity. These proteins will be good candidates for use in the phytoremediation of arsenicals. In addition, because YCF1 activity contributes not only to arsenic, but also to cadmium, mercury, and lead resistance in yeast, our results will likely be applicable toward remediation of several environmental toxicants.

250 Investigation of Vitamin B6 synthesis in Arabidopsis thaliana and its role in abiotic stress response

Elizabeth Rueschhoff, Eugenia Gonzalez, Margaret Daub
North Carolina State University

Vitamin B6 (pyridoxine, pyridoxal, pyridoxamine and their phosphorylated derivatives) is a required cofactor for numerous enzymatic reactions, and has recently been implicated in cellular oxidative stress defense. The de novo pathway for biosynthesis in higher plants differs from the well-characterized pathway in E. coli and has only recently been characterized. In addition to the de novo pathway, all organisms contain a salvage pathway that functions to interconvert between the different vitamers. In plants, two genes are involved in the de novo pathway, PDX1 and PDX2. In Arabidopsis there are three PDX1 homologs; PDX1.1 on chromosome 2, PDX1.2 on chromosome 3 and PDX1.3 on chromosome 5. PDX2 exits as a single copy located on chromosome 5. PDX1 and PDX2 form a complex and function as a glutamine amidotransferase and also carry out the ring closure step in the pathway. Thus far, our lab and others have identified three genes in the salvage pathway; these are genes encoding a pyridoxal kinase (SOS4), a pyridoxine phosphatase/pyridoxamine phosphatase (PNP/PMP) oxidase (PDX3), and a putative pyridoxal reductase. The goal of this work was to investigate the effect of mutations in the Arabidopsis de novo and salvage pathways on oxidative stress responses. Homozygous T-DNA insertion mutants have thus far been recovered for PDX1.2, PDX1.3, PDX3, and the putative pyridoxal reductase. In addition, a sos4 mutant was kindly provided by Dr. Jian-Kang Zhu (University of California–Riverside). Analysis of total B6 levels by HPLC and a yeast bioassay showed that levels were unaffected in the pdx1.2 and pdx3 mutants but were 67% lower in the pdx1.3 mutant. Surprisingly, total B6 levels in the sos4 mutant were 264% of wild type levels. Osmotic and salt stress experiments were carried out with all the mutants. Only the sos4 mutant showed significantly increased sensitivity to salt, with an 88% decrease in root growth when grown on medium containing 100mM NaCl as compared to wild type. In addition, both the pdx1.3 and sos4 mutants showed 77% and 82% decrease in root length, respectively, when grown on medium containing sucrose. None of the mutants showed increased sensitivity to mannitol as compared to wild type plants. Experiments are currently underway to determine if any of the mutants show susceptibility to environmental stresses, such as drought, high light and chilling. We are also making hybrids between mutants to investigate the phenotype and stress responses of lines mutant in multiple B6 biosynthetic genes.

251 Ontogeny of the Arabidopsis Circadian Clock

Patrice Salome, C. Robertson McClung
Dartmouth College, Hanover, (NH), USA

A functional circadian clock allows an organism to synchronize with the daily succession of day and night, and to anticipate changes in the environment. In Arabidopsis, much research has focused on the entrainment of the clock to light-dark (LD) or warm-cold (HC) cycles. Very little is known, however, on the requirements leading to the generation of a rhythm. In zebrafish, prior entrainment is critical for onset of rhythmic behavior. In Drosophila or mammals, entrainment is dispensable for rhythmicity, but not for synchronization with the environment. With the use of the LUCIFERASE reporter gene driven by a number of clock-regulated genes, we set out to investigate the minimal requirements for onset of rhythmic expression in Arabidopsis seedlings. Seedlings grown in constant light were rhythmic, demonstrating that, unlike zebrafish, entrainment is dispensable for rhythmicity. In addition, exposure to light is not a requirement for rhythmicity, as etiolated seedlings displayed rhythms, although with weaker amplitudes, in all genes tested including the LIGHT HARVESTING COMPLEX PROTEIN B (LHCB). Although rhythms were detected in seedlings grown in constant light and constant darkness, the relative synchronization among individual seedlings was not as precise as that seen after LD or HC cycles, especially for the clock-regulated genes CATALASE 3 (CAT3) and LHCB. The observed phase of all genes in the absence of entrainment also appeared to be out of phase with entrained seedlings. Onset of circadian rhythmicity could be observed as early as 1 day after release from stratification, as seen with the expression pattern of the genes PSEUDO RESPONSE REGULATOR 7 (PRR7), CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and TIMING OF CAB 1 (TOC1). Interestingly, rhythmic expression of CCA1, LATE and ELONGATED HYPOCOTYL (LHY) and TOC1 was not detected in germinating, non-stratified seeds until 3-4 days after imbibition, when PRR7 could be readily detected 1-2 days after imbibition. We speculate that germinating seedlings use imbibition to initiate circadian rhythmicity before light exposure. While still buried in the soil, seedlings become synchronized with the outside environment by HC cycles and show a weak amplitude critical for rapid adaptation to new conditions. The first exposure to light will greatly increase the amplitude of circadian oscillations, providing seedlings with high levels of the needed transcripts at this early stage of development.

252 The interaction of the holoparasite, *Cuscuta reflexa*, with *Arabidopsis thaliana*

Dierk Scheel, Mandy Birschwilks
Leibniz Institute of Plant Biochemistry, Halle, Germany

The interaction of *Cuscuta reflexa* with *Arabidopsis thaliana* represents a compatible host–parasite combination. After twinning around the influorescence stem, *Cuscuta* produces a haustorium that penetrates the host tissue. In early stages of development the searching hyphae at the tip of the haustorial cone are connected to the host tissue by interspecific plasmodesmata. Ten days after infection translocation of the fluorescent dyes, Texas red and 5,6-Carboxyfluorescein, demonstrates the existence of continuous connections between xylem and phloem of host and parasite. *Cuscuta reflexa* is the dominant sink in this host-parasite-system. A set of different ecotypes of *Arabidopsis thaliana* is being screened for altered infection phenotypes with *Cuscuta relexa*. One out of 15 ecotypes analyzed so far apparently displays a novel type of resistance against the parasite.

253 Identification and Characterization of a Genetic Locus Controlling Ethylene-Induced Leaf Senescence

Reza Shirzadian Khorramabad, Hai-Chun Jing, Jacques Hille, Paul Dijkwel

Molecular Biology of Plants, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen , The Netherlands

Leaf senescence represents the last stage of leaf development and leads to cell death, thereby limiting the life span or longevity of a leaf. Although senescence occurs in an age-dependent manner, it is known that internal senescence-promoting factors can regulate leaf senescence. In particular ethylene has been shown to be a potent inducer of leaf senescence. In an attempt to better understand the mechanism of ethylene-dependent senescence, we identified and analyzed a mutation, named onset of leaf death 101 (old101). The *old101* mutation extends Arabidopsis leaf longevity and this phenotype is not the result of a reduced sensitivity to ethylene. The segregation analysis of the mutant showed that it is a monogenic recessive trait and the mutation is located on chromosome 5 between BACs K19B1 and MQN23. The old101 mutant showed a delayed onset of various senescence symptoms during ethylene-dependent and age-dependent senescence but little effect on leaf senescence, artificially induced by darkness, was found. The mutation in the OLD101 locus causes a delay in all senescence parameters examined, including chlorophyll content, photochemical efficiency of photosystem II, ion leakage and nutrient remobilization. Relative expression of senescence-associated genes and an ethylene responsive marker gene were significantly lower than the wild type. Expression of photosynthesis-associated genes in old101 leaves was higher than in the wild type. Remarkably, *old101* was more resistant to Pseudomonas syringae pv tomato DC3000 as compared to the wild type, and the stay green mutant seedlings exhibited increased tolerance to oxidative stress. Thus, the *old101* phenotype involves a mutated gene that might encode a protein stimulating the leaf senescence process. An increased understanding of the OLD101 gene function can be important for extending the shelf life of green vegetables and for agronomic improvement and enhanced stress resistance in crop species.

254 Morning-Specific Transcription of an Arabidopsis Clock Gene, LHY

Mark Spensley, Jae-Yean Kim, Isabelle Carre

Department of Biological Sciences. University of Warwick.

LHY and CCA1 encode partially redundant Myb transcription factors that form part of a transcriptional feedback loop which is essential for the function of the Arabidopsis circadian oscillator. Genetic studies have identified several upstream regulators of these genes, including TOC1, ELF3 & LUX, but the mechanisms by which these factors act to regulate the morning-specific transcription of LHY is unclear.

To understand the transcriptional regulation of LHY, we have performed a cis-analysis of the LHY promoter. Rhythmic expression and acute light responses are mediated through a short, proximal region of the promoter, 128bp upstream of the predicted transcriptional start site. An adjacent 103bp of upstream sequence, containing a G-box and a repressive element, modulates the phase of LHY expression and may mediate a second, rhythmic input to the promoter. In vitro analysis of protein-DNA interactions within these two regions has identified four classes of DNA-binding activity that may account for these functions.

We are currently investigating the roles of these activities in the circadian and light regulated transcription of LHY with the aim of understanding how the functions of several upstream regulators are integrated at the LHY promoter to produce the characteristic transcriptional profile of LHY providing greater insight into the functions of LHY and its upstream regulators in the Arabidopsis circadian clock.

255 Cell type-specific expression and boron-dependent endocytosis of BOR1, a boron transporter

Junpei Takano[1], *Kyoko Miwa*[1], *Toru Fujiwara*[1, 2]

[1]Biotechnology Research Center, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan, [2]SORST, JST

Boron (B) is essential for plants and has a structural role in cell wall. Besides its essentiality, B is toxic when present in excess. *Arabidopsis thaliana* BOR1 is a B exporter for xylem loading and is essential for efficient B translocation from roots to shoots under B limitation (Takano et al. 2002). Using transgenic plants expressing BOR1-GFP fusion protein under control of the cauliflower mosaic virus 35S RNA promoter, we have demonstrated that posttranslational mechanisms play a major role in regulation of BOR1 accumulation. In the root tip cells of the 35S:BOR1-GFP plants, BOR1-GFP was localized to the plasma membrane under B limitation and was transferred via the endosomes to the vacuole for degradation upon exposure to high levels of B (Takano et al. 2005).

Here we report cell-type specific expression and boron-dependent endocytosis of BOR1-GFP expressed under control of the *BOR1* promoter. BOR1-GFP was localized to steler cells in mature portion of roots and also to various cells in root elongation zone under B limitation. These results suggest that BOR1 functions in roots are zone specific. BOR1 functions for B transport into xylem in mature portion for root-to-shoot B translocation while it is for radial B transport towards inner portions in root elongation zone. We have recently shown that T-DNA insertion mutants of a boric acid channel *NIP5;1* and a double insertion mutant of B exporters *BOR1/BOR2* were defective in root cell elongation under B limitation (Takano et al. 2006, Miwa et al. unpublished results). Taken together, our results suggest that radial transport of B by these transporters are important for supplying B to elongating cell walls under B limitation.

Moreover, closer observation of epidermal cells in elongation zone revealed that the BOR1-GFP was disappeared from plasma membrane upon exposure to high levels of B. We propose that the B-dependent endocytosis of BOR1 provides a fast and efficient way to control B transport necessary under B limitation but detrimental under high B supply.

Takano et al. (2002) ***Arabidopsis*** boron transporter for xylem loading. Nature 420: 337-340

Takano et al. (2005) Endocytosis and degradation of BOR1, a boron transporter of ***Arabidopsis thaliana***, regulated by boron availability. PNAS 102: 12276-12281

Takeno et al. (2006) The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. Plant Cell, 18 (6)

256 Functional analysis of metal transporters in Thlaspi caerulescens

Sangita Talukdar, Mark Aarts

Wageningen University

Heavy metal hyperaccumulation in plants is a poorly understood phenomenon. Transmembrane metal transporters are assumed to play a key role in this process. In our research Zn transporters of Thlaspi caerulescens, a heavy metal hyperaccumulator plant, are studied and compared to orthologues of Arabidopsis thaliana, a non-hyperaccumulator plant. The ZTP1 Zn transporter gene shows 85% sequence similarity in the coding region with the ZAT/MTP1 gene of Arabidopsis and is assumed to be localized in the vacuolar membrane and suggested to function in vacuolar metal loading. This gene belongs to the Cation Diffusion Facilitator (CDF) family. The ZNT1 and ZNT2 genes are members of the Zrt, Irt (ZIP)-like gene family and show 89% & 87% similarity with the ZIP4 and IRT3 genes of A. thaliana, respectively. Constitutively high expression of ZNT1 and ZNT2 in roots, irrespective of the Zn concentration in the medium whereas the A. thaliana ZIP4 and IRT3 genes are induced exclusively by Zn-deficiency, suggests a role for these genes in Zn uptake. The proteins are assumed to be localized in the plasma membrane, conferring zinc uptake into the cytoplasm. 35S promoter -ZNT1 and -ZNT2 showed higher sensitivity and early flowering when grown on low Zn, than wild type Columbia. 35S promoter - ZTP1 transgenic plants shows higher tolerance to high Zn compared to wild type. The response of these genes to different concentrations of divalent metal ions (Zn, Cd, Mn, Fe and Cu) and the complementation of A. thaliana mutants by T. caerulescens genes will be checked. Preliminary studies of transiently expressed ZNT1-GFP and ZNT2-GFP constructs in cowpea protoplasts indicated localization in the plasma membrane. The regulation of expression of these genes is studied in comparison to the orthologous genes in A. thaliana. In order to examine the response of the ZIP4 promoter to different Zn media, transformed Arabidopsis plants with a ZIP4 promoter::GUS construct were studied, which shows induction by low Zn only. The ZNT1 promoter was isolated by PCR using forward primers designed on the A. thaliana gene upstream of ZIP4 and a reverse primer on the T. caerulescens ZNT1 cDNA (of which the upstream gene is unknown). The promoters of ZIP4 were isolated in a similar method from Arabidopsis halleri, Arabidopsis lyrata and the related Cochlearia pyrenaica. The expression of the ZIP4 genes of all these five species in response to different Zn concentrations, will be studied by quantitative RT-PCR.

257 Two NRT1 (PTR) Genes, When Overexpressed in Roots, Exhibited Cd-sensitive Phenotype

Chyn-Bey Tsai, Yi-Fang Tsay

Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan

To date, there are only few of the 53 Arabidopsis NRT1 (Nitrate Transporter family 1 or PTR, Peptide Transporter family) family members being characterized including 3 nitrate transporters (AtNRT1:1, AtNRT1:2 and AtNRT1:4) and 2 peptide transporters (AtPTR2 and AtPTR1). Therefore, the in vivo functions of the majority in this gene family remained unclear. To investigate physiological functions of these homologs, we took reverse genetic approach and established a cadmium sensitivity assay by comparing the root elongation between the T-DNA insertion mutants of 53 NRT1 genes and wild type. Interestingly, mutants of At3g01350 and At5g14940 were found to be cadmium sensitive. At3g01350 and At5g14940 are redundant genes due to the Chromosome duplication event. Quantitative real time PCR reveals that the cadmium-sensitive phenotype was correlated with overexpression of either At5g14940 or At3g01350 in roots. Two-electrode voltage clamping analysis of *Xenopus* oocytes expressing At5g14940 indicates that, in addition to nitrate, this transporter is capable to transport glutathione, and γ-EC which are precursors of phytochelatins used for plant cadmium detoxification. In this study, we identify new substrates, glutathione and γ-EC, and new physiological role for the NRT1 (PTR) family.

258 *Arabidopsis* Mitochondrial Prohibitin AtPHB4 Regulates A Stress Regulon Involved In Auxin Homeostasis

Olivier Van Aken[2], Kris Morreel[2], Brigitte van de Cotte[2], Hillel Fromm[1], Wout Boerjan[2], Dirk Inze[2], Frank Van Breusegem[2]

[1]Department of Plant Sciences, Tel Aviv, Israel, [2]Plant Systems Biology - VIB2, Ghent, Belgium

Prohibitins are evolutionarily conserved proteins with diverse roles in eukaryotic cell cycle progression, mitochondrial electron transport, cellular signaling, aging and apoptosis. We demonstrate that prohibitins of *Arabidopsis thaliana* are targeted to the mitochondria and are primarily expressed in apical tissues and dividing cells. Overexpression of *AtPHB4* in transgenic *Arabidopsis* plants leads to increased shoot branching and leaf shape aberrations. A genome-wide microarray analysis of $AtPHB4^{OE}$ transgenic plants revealed differential expression of a confined regulon of 34 transcripts. We infer from a microarray meta-analysis that this regulon is strongly co-regulated during abiotic stress conditions and show that one of its genes, a putative UDP-glucosyltransferase *UGT74E2*, is a novel indole-3-butyric acid glucosyltransferase that is involved in the increased shoot branching of $AtPHB4^{OE}$. We suggest that increased UGT74E2 activity steers auxin catabolism in the apical tissues under unfavorable growth conditions and propose that AtPHB4 provides a previously unidentified mitochondrial interface between stress perception and auxin homeostasis.

259 Characterization of Flowering Time Responses to Photoperiod and Temperature in Diverse Ecotypes of *Arabidopsis thaliana* and its Modification by the Gene *CO*

Daniel Villegas, Jose Alcalde

Facultad de Agronomia e Ingenieria Forestal. Pontificia Universidad Catolica de Chile. Casilla 306-22 Santiago, Chile.

Flowering time is the most important phenological event in plant life because determines adaptation to environment. Among several environmental factors that affect flowering time, temperature and photoperiod are the most important, plants show genetic variation in the responses to them. Roberts and Summerfield (1987) proposed a simple **Linear Photothermal Model** to predict flowering time (***f***) in which rate of progress to flowering (i.e. the inverse of ***f*** or 1/***f***) is a linear and additive function of average **T** and/or **P** from sowing to flowering. This model has been shown to fit well to several crop plants (peas, soybean, etc) allowing quantification of the effects of different genes that modify the responses of the rate of progress to flowering to **T** and **P**, and their influence on adaptability to different environments. This association between gene effects on flowering and the parameters of the linear photothermal model allowed quantitative biological-functional interpretation of the model. First, we tested this model on different ecotypes of *Arabidopsis* (representing wide range from early to very late types) to know if their responses can be adequately represented by the model; and second, considering the advances in understanding the molecular aspects of flowering control in this species, we studied the effect of genes that may play a role in modulating control the flowering in *Arabidopsis* such CONSTANS, to determine if this gene has a major modifying effect on specific parameters of the model. To do this, we create twelve different photothermal environments covering a 10 to 25 °C temperature range and a 10 to 20 h d^{-1} photoperiod range. All ecotypes were grown in these different environments and days from sowing to flower, number of leaves at bolting and number of leaves at flowering were registered. The results show that in all the ecotypes the linear photothermal model described well the response of rate of progress to flowering, defining at least two planes of response (Thermal and Photothermal planes). With these results, time to flowering under any photothermal environment covered by this study can be accurately predicted. Ecotype Wassilewskija was the earliest to flower (33 d at 20 ^{o}C and 20 h d^{-1}) while Zurich shows no flower response after 120 d at 14^{o}C and 10 h d^{-1}.

Research funded by FONDECYT, Chile. Project No 1040551. A CONICYT Scholarship for doctoral studies for D.V. is gratefully acknowledged.

260 Sumoylation facilitates basal thermotolerance in Arabidopsis through salicylic acid–independent processes

Chan Yul Yoo[1], Kenji Miura[1], Jing Bo Jin[1], Jiyoung Lee[2], Dae-Jin Yun[2], Ray Bressan[1], Paul Hasegawa[1]

[1]Center for Plant Environmental Stress Physiology, Purdue University, [2]Division of Applied Life Science (BK21), Gyeongsang National University

AtSIZ1 is a SUMO (small ubiquitin modifier) E3 ligase that is an ortholog of PIAS-type proteins, which facilitates SUMO conjugation to substrate target proteins (sumoylation) in Arabidopsis (Arabidopsis thaliana). siz1 T-DNA mutations (siz1-2 and siz1-3) cause basal, but not acquired, thermosensitivity that is associated with hyper-accumulation of salicylic acid (SA). Expression of NahG, which encodes a salicylate hydroxylase, effectively reduces endogenous SA accumulation but enhances thermosensitivity resulting from siz1-2. High temperature induces SUMO1/2 conjugation to peptides in wild type, but to a substantially lesser degree in siz1 mutants. In other organisms, heat shock transcription factor (HSF) sumoylation regulates DNA binding and activation of heat shock protein (HSP) gene expression that facilitates thermal adaptation. However, heat shock–induced expression of genes, including HSPs, APX1 and APX2, is similar in siz1 and wild-type seedlings, further indicating that SIZ1 does not regulate acquired thermotolerance. Together, these results indicate that sumoylation through SIZ1 facilitates basal thermotolerance through processes that are SA independent and supersedes negative regulation of SA accumulation by the E3 ligase.

261 The Role of SHB1 in Photomorphogenic Development and Photoperiodic Flowering

Yun Zhou, Xiaodong Sun, Min Ni
Department of Plant Biology University of Minnesota, Twin Cities

The red, far-red, and blue light perception and signaling regulate photomorphogenic development, photoperiodic flowering, and circadian rhythm. We have identified an Arabidopsis mutant shb1 for short hypocotyl under blue 1, a knockout allele. However, shb1-D, a dominant overexpression allele, exhibits a long hypocotyl phenotype under red, far-red, and blue light. Therefore, SHB1 is involved in cry-mediated blue light signaling, and overexpression of SHB1 may expand its signaling activity to red and far-red light. We have since undertaken deletion analysis to define the functional domains of SHB1 in transgenic Arabidopsis. The transgenic plants that overexpress its N-terminal 520 amino acids phenocopied shb1-D with a long hypocotyl phenotype under red, far-red, and blue light. The N-terminal truncation has a SPX motif homologous with SYG1 protein family members, and the SPX domain is apparently critical for SHB1 function. In contrast, the transgenic plants that overexpress three C-terminal truncations resembled shb1 with a short hypocotyl under blue light. The phenotypes may be created through a dominant negative mechanism, and all three C-terminal truncations contain a putative EXS domain found in the SYG1 protein family. We have further conducted genetic screens for suppressors and enhancers of shb1-D and worked toward the identification of genes that genetically interacts with SHB1. shb1-D also flowered early under long days but the same as wild type or slightly early under short days. SHB1 may mediate the photoreceptor regulation on the expression of several key flowering genes, and may define a new signaling step in the regulation of both photomorphogenic development and photoperiodic flowering. Future studies on SHB1 will reveal the critical crosstalk and integration steps of blue light with red and far-red light signaling branches.

262 ADP-Ribosylation in Plant Disease and Plant Disease Resistance

Lori Adams-Phillips, Andrew Bent
University of Wisconsin-Madison

We have discovered that ADP-ribosylation processes are part of the plant defense response. ADP-ribosylation is the transfer of ADP-ribose from NAD^+ onto specific sites on target proteins, causing activation or inactivation of those proteins. This is a new, largely unstudied area of plant pathogenesis. However, ADP-ribosylation is very well known in the mammalian pathogenesis field, both from the causal role of bacterial ADP -ribosyltransferases in cholera, pertussis and diptheria diseases, and from host regulation of cellular stress, inflammation and cell death through modulation of ADP-ribosylation. A number of independent lines of evidence reveal the involvement of ADP-ribosylation during plant/host interactions. We have discovered that genes encoding a plant ADP-ribose pyrophosphatase and a plant poly(ADP-ribose) glycohydrolase are among the 39 most reliably up-regulated genes during the Arabidopsis defense response. In addition, we have determined that levels of poly(ADP-ribose) increase substantially during compatible interactions with Pseudomonas syringae and before the onset of HR cell death during the RPS2-mediated defense response. Furthermore, plants mutated in the upregulated ADP-ribose pyrophosphatase gene exhibit a reduced HR and increased resistance to Pseudomonas syringae. Plant lines have been constructed that carry either constitutive or inducible over-expression constructs, or gene silencing constructs for genes involved in ADP-ribosylation. Pharmacological inhibitors of ADP-ribosylation are also being utilized to identify the aspects of plant disease resistance where the observed shifts in ADP-ribosylation activities exert a significant impact.

263 Involvement of Cytokinins and Two-Component Elements in Arabidopsis responses to pathogens

Cristiana Argueso, Joseph Kieber
University of North Carolina at Chapel Hill

Cytokinins are plant hormones involved in many aspects of plant biology and development, such as regulation of cell division, senescence and sink-source relationships within the plant. While the plant hormones salicylic acid, jasmonic acid and ethylene have been implicated in the regulation of plant defense responses against pathogens, the role of cytokinins remains unclear. Recent studies have revealed that cytokinin signaling involves a phosphorelay pathway similar to two-component element response systems, used by bacteria and other organisms to sense and respond to a diverse array of environmental stimuli. We have tested Arabidopsis mutants with T-DNA insertions in genes encoding two-component elements, as well as transgenic lines, for their susceptibility to biotrophic and necrotrophic pathogens. Some of these mutants show increased or decreased susceptibility to pathogens, indicating a possible role for two-component elements and cytokinins in Arabidopsis responses to pathogens. It is possible that cytokinins/ two-component elements could play a role in plant-pathogen interactions either through the activation of plant defense responses or through the regulation of sink-source relationships within the plant. Our research may help elucidate additional roles of cytokinins in plant biology, as well as contribute to a better understanding of plant-pathogen interactions and to the generation of plants with increased disease resistance.

264 Identification of infection-related proteins at the host-pathogen interface of Arabidopsis

Nico Boot[1], Monique Slijper[2], Albert Heck[2], Guido van den Ackerveken[1]
[1]Molecular Genetics Group, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands, [2]Biomolecular Mass Spectrometry, Department of Chemistry and Department of Pharmaceutical Sciences, Faculty of Science, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands

The host cell plasma membrane (PM) constitutes an important barrier that restricts extracellular pathogens from having access to the cytoplasm of the host cell. Being the site of contact between host and pathogen, the PM plays a key role in nutrient exchange and signal transduction between the interacting organisms. To proliferate extracellularly pathogens need to suppress host cell defenses and manipulate nutrient metabolism and transport. To achieve this the bacterial pathogen Pseudomonas syringae pv. tomato (Pst) uses its type III secretion system to inject a plethora of effector proteins into the host cell cytoplasm. Several of these effectors have been shown to suppress plant defense. However, the influence of Pst on signaling and transport processes over the PM of the host is unknown. We hypothesize that proteins involved in these processes are more abundant at the PM during infection. To elucidate the molecular processes occurring at the host-pathogen interface of Arabidopsis (accession Col-0) with virulent Pst (DC3000 strain) we have isolated PM of mock-treated and Pst-infected Arabidopsis seedlings. Tryptic digests of the PM fractions were subjected to semi-quantitative LC-MS/MS analysis. We have identified several proteins that show differential presence in Pst-infected vs. mock-treated PM fractions. Our data indicate that several proteins become PM-associated during the infection process while their overall protein levels do not increase. Functional studies will elucidate if these PM-associated proteins play an important role during the infection process.

265 Translesion polymerases influence growth rate in the presence of DNA lesions

Marc Curtis, John Hays
Oregon State University

DNA damage induced by UV-B light, reactive oxygen species, or other agents threatens plant genomic integrity. DNA lesions present during S-phase can block replication. Persistent collapsed replication forks can give rise to lethal double-strand breaks. Specialized translesion polymerases (TLPs) with inherently low fidelity synthesize DNA past template lesions, with relatively 'error-free' or 'error-prone' results. Although all TLPs lack proofreading exonucleases, the extent of errors depends on the particular TLP or combination of TLPs involved, and the particular template lesion being bypassed. Four TLPs are conserved across eukaryotic kingdoms. Pol κ, Pol η and Rev1 are members of the Y-family of DNA polymerases. The fourth conserved eukaryotic TLP is Pol ζ, a member of the B-family of DNA polymerases, which includes the major replicative (proofreading) polymerases. Unlike the high fidelity replicative polymerases, Pol ζ is able to elongate distorted primer-template termini that arise at DNA lesions.

In root assays, mutant Arabidopsis lacking Pol η (AtRAD30-1/1) are sensitive to UV-B, exhibiting a transient inhibition of growth rate. Plants lacking Pol ζ (AtREV3-2/2) are more sensitive to UV-B, perhaps due to a cell cycle arrest. Plants lacking both polymerases show a prolonged UV-B-induced inhibition of growth, perhaps due to irreparable damage to initial cells and some death of initials; recovery appears to occur by replacement of initial cells from the quiescent center. In double mutants, sensitivity was more than additive, suggesting inter-dependent functions. The two major UV photoproducts are cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6,4) pyrimidone dimers; Pol η is known to bypass the former efficiently but not the latter. Bypass of the 6,4 photoproduct requires Pol ζ, which also may extend primer termini across from CPDs that are in sequence contexts problematic for Pol η.

TLPs also influence the coordinated growth of ovule and gamete development (absent UVB): in AtREV3-2/+ heterozygotes, 50% of the female gametes are buried in the integuments. Moreover, progeny of selfed AtREV3-2/+ AtRAD30-1/+ double heterozygotes exhibit non-Mendelian segregation. Surprisingly, single heterozygotes, double mutants, and wild-type progeny were all underrepresented, consistent with selective loss of wild-type and double-mutant gametes. (Supported by NSF grant MCB-0345061 to J.B.H.)

266 Functional evaluation of plant defence signalling against Fusarium ear blight disease in Arabidopsis

Alayne Cuzick, Kerry Maguire, Sarah Lee, Kim Hammond-Kosack
Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

Fusarium ear blight (FEB) infections of cereal crops cause considerable losses to grain quality and safety. The two main causative agents of this disease on wheat crops are the hemibiotrophic pathogens F. culmorum (Fc) and F. graminearum (Fg) (sexual stage Gibberella zeae). Floral infections by Fc and Fg also cause the developing cereal grains to become contaminated with various fungal mycotoxins, including the highly toxic trichothecene mycotoxin deoxynivalenol (DON). The molecular basis of resistance to FEB in cereal species is poorly understood but it is QTL based and Fusarium species non-specific.

We have previously demonstrated that Fc and Fg conidia can infect the floral tissues of Arabidopsis to cause disease symptoms on flowers, siliques and upper stem tissue [1]. DON mycotoxin production was detected in infected flowers. This novel Arabidopsis floral model provides a tractable system for elucidating fundamental aspects of this globally important cereal-fungal interaction.

We are now undertaking a detailed analysis of the FEB infection phenotype in Arabidopsis genotypes with defined gene mutations. Data will be presented on the effects of genes involved in basal and race-specific defence signalling, including RAR1, SGT1 and EDS1. Infections are quantified by FAD values (Fusarium-Arabidopsis Disease) [1].

Forward genetics experiments are also being conducted to select EMS mutagenised Arabidopsis lines resistant to DON mycotoxin.

We are in the process of obtaining / generating a selection of transgenic fusarium strains that contain different reporter contructs. These include constitutive expression of GFP (green flurorescent protein) or GUS (ß-glucuronidase) as well as *TRI5* promoter mediated GUS expression to mark the onset of mycotoxin production. These strains will be used to aid the visualisation and understanding of the Fusarium-Arabidopsis pathosystem in both wild-type and mutant genotypes.

This research is supported by the Biotechnology and Biological Sciences Research Council of the UK .

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267 Identification of new actors of the hypersensitive cell death in Arabidopsis using AtMYB30 as a starting point.

Xavier DANIEL, Dominique ROBY
LIPM CNRS/INRA Toulouse

In an incompatible interaction, the most spectacular plant response is the Hypersensitive Response (HR), characterized by the plant cell death at the site of the attempted infection by the pathogen. Few data are available on the regulators of this cell death genetic program, tightly linked to plant resistance to pathogens. To identify such regulators, two approaches have been developed in the lab: isolation of genes specifically induced during the HR (differential screenings), and isolation of genes which mutation leads to an altered HR (screens for mutants displaying a lesion mimic phenotype or enhanced disease/ susceptibility phenotype). *AtMYB30* was identified by differentially screening a cDNA library generated from Arabidopsis cell suspensions grown in the presence of cycloheximide and inoculated by *Xanthomonas campestris* pv. *campestris*. AtMYB30 protein contains a domain displaying strong homologies to other plant and animal MYB proteins. Analysis of *AtMYB30* expression during diverse interactions shows that maximal transcription clearly precedes the HR (1). *AtMYB30* expression is deregulated in Arabidopsis mutants affected in the control of the hypersensitive cell death, suggesting a strong correlation between its expression and the initiation of the hypersensitive cell death. To get further insight into the role of AtMYB30 in this program, Arabidopsis and tobacco transgenic lines over-expressing *AtMYB30* in sense (AtMYB30ox) and antisense orientations were generated. Their response to avirulent and virulent bacterial and fungal pathogens display a modification of the kinetics and intensity of the HR, as well as of disease resistance. These data demonstrate the positive regulator role of AtMYB30 in the initiation of the hypersensitive cell death in response to pathogen attack (2). However, the absence of spontaneous lesions in the AtMYB30ox plants, the altered resistance phenotype to avirulent pathogens, and the profound modification of their susceptibility to virulent pathogens (HR-like phenotype) show that AtMYB30 cannot act by itself. AtMYB30, being a positive regulator of the hypersensitive cell death and resistance in Arabidopsis in tobacco, and needing other factor(s) to control the genetic program associated with the HR, is being used as a starting point for the identification of new actors of this program. The methods used for this purpose, and the first identified candidates are presented.

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268 Relating Pathogen Infection and Host Genotype in Midwestern Arabidopsis Populations

Megan Dunning, Joy Bergelson
University of Chicago

The interaction between plants and pathogens is believed to be an important force shaping the ecology and evolution of plant populations. For this to be true in a natural plant-pathogen system, host resistance and pathogen virulence must vary according to host genotype. To test this prediction, leaf samples have been collected from *Arabidopsis thaliana* populations in Indiana and Michigan. The growth of three known bacterial pathogens found in these fields (*Pseudomonas viridiflava, P. syringae* and *Xanthomonas campestris*) was recorded in the collected leaves in order to assess pathogen load. In the host, a set of SNP markers distributed across the genome has been selected to assess the genome-wide haplotype for each *A. thaliana* plant sampled. By comparing naturally occurring pathogen load with host genotype, we can ask whether pathogen growth is correlated with host frequency and relative growth of other pathogens.

After assessing the growth of these three pathogens in *A. thaliana* leaves, both individuals and local populations show differences in infection prevalence and severity. Although these *Arabidopsis* populations have been previously shown to have low genetic diversity compared to a worldwide sample, our preliminary genotyping suggests that previous studies may have underestimated the genetic diversity in Midwestern populations. Nonetheless, several haplotypes are shared among populations, allowing us to compare pathogen load across genetically identical individuals. By examining the frequency and abundance of these pathogens in Midwestern populations of *A. thaliana*, we hope to clarify the importance of host genotype for disease resistance in the field.

269 A Genetic Analysis of Oxylipin Roles in Cell Survival

Laurent Mene-Saffrane, Stephanie Stolz, Edward Farmer
University of Lausanne

Numerous genetic studies have confirmed the importance of the canonical jasmonate pathway in plant defense. However linolenic acid (18:3), the source of jasmonates, can be converted in vivo into many other oxylipins. Many of these are made enzymatically, but non-enzymatic oxidation of 18:3 is known to occur in vivo even in healthy tissues. Normally, non-enzymatic lipid oxidation is tightly managed by the cell but in some circumstances there is loss of control over the process. This can occur in some severe stresses and in some pathosystems where the production of excess reactive oxygen species (ROS) can lead to the oxidative damage. 18:3 oxidation generates of a large spectum of molecules many of which are regarded as molecular junk and for which biological roles have not been investigated. Among these molecules are many 'reactive electrophile species' (RES), molecules potentially capable of affecting gene expression in the host (and pathogen). To investigate the roles of 18:3 and its products in cell survival we first combined lesion mimic mutants with plants lacking the ability to produce all jasmonates. In parallel, we investigated the effects of 18:3 removal from lesion mimic mutants. These plants displayed very different phenotypes to those lacking jasmonates with greatly accelerated lesion development. 18:3 itself or products from this molecule strongly retard lesion formation in the absence of jasmonates. The results suggest that oxylipin signaling extends to molecules outside the jasmonate family and that multiple 18:3-derived compounds may play signaling roles in severe stress.

270 Fungal pathogens of man are also pathogens of Arabidopsis

Ines Arrieta-Aguirre, Francisco Fierro Fierro, Enrique Ritter, Susana Garcia-Sanchez
NEIKER

Candida genus of fungi comprise significant pathogens of man. *Candida albicans* is responsible for most opportunistic infections in immuno-compromised patients (AIDS or cancer-treated patients). The related species *C. glabrata*, *C. tropicalis* or *C. dublinensis* are emerging pathogens that are transmitted as nosocomial agents in hospitals. Interestingly these fungi were first isolated in soil samples, and it is only recently that their importance as human pathogens is being reported. Since plants are in constant and intimate contact with soil-borne microbes they have evolve several defence mechanisms that could be useful in the fight against potential, ubiquitously-distributed pathogens. These include the production of antibiotic substances, the recognition and neutralisation of microorganisms in specialises structures, or the development of mutuality relationships with microbia.

Here we used the plant *Arabidopsis thaliana* as a model host for studying *Candida* infections. Plants were grown in liquid medium at 30ºC and inoculated with 10 different species of *Candida* isolated from human hosts. *C. tropicalis, C. kefyr, C. parapsilopsis* and *Issatchenkia occidentalis* produced severe disease symptoms 2 days post-infection, with plants showing chlorosis, tissue degeneration and a increase of fungal planktonic growth in the medium. In contrast, *C. albicans* CAI4, *Scedosporium prolificans* and *C. lusitanicae* did not cause severe damage to he plants and did not increased planktonic growth, but formed a well-developed biofilm on the plat roots. Thus the ability to establish biofilms on the root surface correlates with the survival of the plant, as a reminiscence of mycorizal formation. To study the *Arabidopsis* response to these fungi, a strategy for high-throughput screening of plant proteins able to bind fungal cells is discussed.

271 Scarecrow-like Protein SCL14 Interacts with TGA Factors and Functions as an Essential Co-activator of as-1-dependent Transcription

Tanja Siemsen, Corinna Thurow, Benjamin Fode, Ralf Weigel, Christiane Gatz
University of Goettingen, Untere Karspuele 2, 37073 Goettingen, Germany

Plant defense signalling molecule salicylic acid (SA) as well as xenobiotic stress cues like 2.4D (dichlorphenoxy acetic acid) and the herbicide paraquat induce a number of defense genes containing the *cis* regulatory element *activation sequence-1* (*as-1*). *As-1* type *cis* elements are bound by basic/leucine zipper (bZIP) transcription factors of the TGA family. In order to identify factors regulating TGA activity, a yeast protein interaction screen with *Arabidopsis thaliana* TGA2 as bait and an *Arabidopsis thaliana* cDNA library was performed and led to the identification of SCARECROW-LIKE protein 14. Functional analysis in *scl14* knock out plants revealed that SCL14 is required for the expression of the "truncated" *CaMV 35S* promoter, which contains the *as-1* element as the only regulatory sequence. Chromatin immunoprecipitation (ChIP) experiments revealed 2.4D-enhanced association of SCL14 and TGA2 to the *as-1* element *in planta*. As the activity of known *as-1*-containing promoters like the *GST6* and the *PR-1* promoter was not altered, a whole genome microarray analysis was carried out to identify endogenous promoters that required SCL14 for expression. Two genes that were down-regulated in the *scl14* mutant were further analysed. ChIP analysis showed recruitment of SCL14 and TGA2 to these promoters. In the *tga2tga5tga6* background, SCL14 showed no *in vivo* binding to its target promoters, supporting the model that SCL14 is tethered to the promoter by its interaction with *as-1*-bound TGA factors, where it functions as a transcriptional co-activator.

272 Signalling Pathways Involved in the "defense, no death" Phenotypes of Arabidopsis Mutants dnd1 and dnd2

Ruth Genger[2], Grace Jurkowski[1], John McDowell[3], Andrew Bent[2]
[1]Department of Oncology, University of Wisconsin-Madison, Madison WI 53706, [2]Department of Plant Pathology, University of Wisconsin-Madison, Madison WI 53706, [3]Fralin Center for Biotechnology, Virginia Tech, Blacksburg VA 24061

Plant disease resistance pathways have as key mediators the signaling molecule salicylic acid (SA) and the two plant hormones ethylene and jasmonic acid. Numerous studies have demonstrated various levels of antagonism between these defense signaling pathways, indicative of crosstalk. The Arabidopsis "defense, no death" (dnd) mutants dnd1 and dnd2(hlm1) exhibit constitutive activation of plant defenses (including expression of defense marker genes), elevated SA levels, and enhanced resistance to biotrophic and hemibiotrophic pathogens. In addition, they exhibit no, or a greatly reduced, hypersensitive response to avirulent pathogens. These phenotypes result from mutations in two separate cyclic nucleotide-gated ion channels, AtCNGC2 or AtCNGC4 respectively. We generated and characterized double and triple mutants in the dnd1 and dnd2 genetic backgrounds using well-characterized mutant alleles of NPR1, SID2 (EDS16), NDR1, and EIN2 to evaluate in greater detail the contributions of the wild-type genes DND1 and DND2/HLM1 with regard to known defense signaling cascades. We found that both SID2 and NPR1 were required for resistance of dnd1 and dnd2 to virulent and avirulent Pseudomonas syringae and virulent Hyaloperonospora parasitica, indicating a requirement for signaling through SA. Although mutation of NPR1 did not affect SA levels in dnd1, SID2 was required for increased SA levels in dnd1. EIN2 was not required for resistance of dnd1 or dnd2 to P. syringae or H. parasitica, or for increased SA levels in dnd1. Mutation of ein2 compromised resistance of dnd1 to the necrotroph Botrytis cinerea. NDR1 was required for resistance of dnd1 and dnd2 to avirulent P. syringae expressing avrRpt2, and resistance of dnd1 to virulent H. parasitica. However, for resistance to virulent P. syringae, NDR1 was required in the dnd2 background, but not in the dnd1 background, indicating a rare difference in defense signaling between dnd1 and dnd2. NDR1 was required for increased SA levels in the dnd1 background. At the molecular level, we noted that npr1 and sid2 mutations reduced/eliminated constitutive PR-1 gene expression of dnd1 plants, but intriguingly induced PDF1.2 expression. This suggests that the defense signaling activated by DND1 or DND2 mutations uses SA-mediated NPR1-dependent pathways as the default pathway, but when SA or NPR1 are not available, defense signaling is diverted toward PDF1.2 expression. None of the double mutants showed restoration of the loss-of-HR phenotype, indicating at least partly separate control of the HR from the other phenotypes of dnd1 and dnd2.

273 The Cytochrome P450 Monooxygenase CYP71A13 is Required for Camalexin Synthesis in Arabidopsis

Jane Glazebrook, Christopher Botanga
Department of Plant Biology, University of Minnesota

Camalexin, a phytoalexin produced by Arabidopsis, plays an important role in resistance to the necrotrophic fungal pathogen *Alternaria brassicicola.* The biosynthetic pathway for camalexin biosynthesis is not completely understood. Tryptophan is converted to indole acetal oxime by cytochromes P450 CYP79B2 and CYP79B3. A double *cyp79B2 cyp79B3* mutant fails to produce camalexin, indicating that indole acetal oxime is a precursor to camalexin as well as to indole glucosinolates. *PAD3* encodes CYP71B15, and *pad3* mutants also fail to produce camalexin. CYP79B15 presumably acts downstream from indole acetal oxime, as production of indole glucosinolates are unaffected by *pad3* mutations. Here, we show that CYP71A13 is also required for camalexin synthesis. Plants with cyp71A13 mutations produce greatly reduced amounts of camalexin after infection by either *Pseudomonas syringae* or *Alternaria brassicicola*. Like *pad3* mutants, *cyp71A13* mutants show increased susceptiblity to *Alternaria brassicicola,* but not to *Pseudomonas syringae.* The *cyp79B2 cyp79B3* double mutant also shows these phenotypes. Like *PAD3,* CYP71A13 is expressed at very low levels in uninfected plants, and is rapidly and strongly indluced in response to infection. *PAD3* and *CYP71A13* expression levels are strongly correlated. We conclude that CYP71A13 carries out a reaction in the camalexin biosynthetic pathway, most likely acting downstram from production of indole acetal oxime.

274 Functional Genomics of Arabidopsis Defense Responses Against Pseudomonas syringae

Lin Wang, Jane Glazebrook
University of Minnesota, Department of Plant Biology

Plant defense against bacterial pathogens is a complex and sophisticated system under the control of numerous genes. Understanding how these genes function and how signals are transduced in the disease signaling network will be potentially beneficial for enhancing disease resistance in crop species. A relatively high-throughput method for studying functions of genes that are involved in Arabidopsis defense responses against Pseudomonas syringae has been developed. Genes that were significantly up-regulated after P. syringae infection were chosen as our candidates. Homozygous T-DNA insertion mutants of these target genes were then studied to determine whether or not they have enhanced disease susceptibility (eds) phenotypes. So far, mutants with defects in more than 10 candidate genes have been found to have eds phenotypes. We are now characterizing the eds mutants using a custom microarray to determine whether or not they have defects in defense signaling. The results of these experiments will be presented.

275 Protein Prenylation Is Required In Plant Innate Immunity

Sandra Goritschnig[1], Yuelin Zhang[2], Xin Li[1]

[1]Michael Smith Laboratories and Dept. of Botany, University of British Columbia, Vancouver, Canada, [2]National Institute of Biological Sciences, Beijing, People's Republic of China

Plant defenses toward invading pathogens involve intricate signaling networks that fine-tune physiological responses in the infected cells to limit pathogen spread while minimizing harmful effects on the rest of the plant. Specific resistance responses are mediated by Resistance (R-) proteins that recognize pathogen-derived molecules and initiate signaling cascades culminating in a successful resistance response.

We use a unique gain-of-function *R*-gene mutant, *snc1*, as a tool to identify and study components of resistance signaling in Arabidopsis. In *snc1* a point mutation in an RPP5 homolog causes constitutive expression of pathogenesis-related genes and enhanced resistance to virulent pathogens. In a screen for suppressors of snc1-mediated constitutive resistance, we identified a number of *modifier of snc1* (*mos*) mutants that reveal roles for nucleo-cytoplasmic trafficking (1, 2) and RNA metabolism (3) in defense signaling.

Here, we present *mos8*, another suppressor of *snc1* that completely disrupts resistance against virulent bacterial and oomycete pathogens. *mos8* was found to be allelic to *enhanced resistance to abscisic acid 1* (*era1*), a mutant in the beta-subunit of plant farnesyltransferase, which was first described based on phenotypes correlating with ABA signaling, including germination and stomatal closure. *era1* mutants also have enlarged meristems and flower defects, indicating a role for prenylation in meristem identity.

mos8 and other *era1* alleles show enhanced susceptibility to several virulent pathogens, indicating a requirement for prenylation in basal resistance. Responses to avirulent pathogens are partially affected in *mos8*, further suggesting the existence of highly divergent and pathogen-specific signaling pathways, some of which require prenylated proteins. Using *mos8* and a reverse genetics approach we are addressing potential functions for prenylated proteins in plant resistance signaling. Our research provides insight into novel aspects of the interplay between protein modification and resistance signaling pathways.

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276 A toolkit allowing induction of IAA and indole glucosinolate production *in planta*

Bjarne Hansen, Jing LI, Ida Soenderby, Niels Jensen, Barbara Halkier

Department of Plant Biology, Plant Biochemistry laboratory, KVL, Copenhagen, Denmark

Characteristic for the cruciferous plants, which include Arabidopsis, is the content of a variety of sulphur-rich indole compounds, such as e.g. indole glucosinolates and indole alkoloids, e.g. camalexin in Arabidopsis. These compounds are known to play a role in plant defence either as constitutively expressed phytoanticipines or as pest-inducible phytoalexins. Furthermore, the breakdown product of specific glucosinolates has been shown to exhibit surprisingly potent but well-documented cancer-preventive properties.

Recent findings have demonstrated that IAOx constitutes an important branching point between the biosynthetic pathway of indole glucosinolates, IAA, and camalexin in the model plant Arabidopsis.

We have reintroduced CYP79B2 under the control of the ethanol-inducible promoter AlcA in a cyp79B2/cyp79B3 double knockout and in a cyp79B2/cyp79B3/cyp83B1 triple knockout mutant, which allows the control of IAOx biosynthesis. The control of IAOx production has been found to be very tight with no IAOx production in the absence of ethanol and a high level of IAOx production after ethanol treatment. The two genetic backgrounds allow the induction of either indole glucosinolate or IAA production. This provides a powerful system for investigating the role of indole glucosinolates and camalexin in plant defence. Furthermore, it provides a system that allows the use of microarray for gene discovery of auxin responsive genes and IAOx-metabolizing enzymes as these plants are morphological identical before treatment with ethanol.

Premilinary microarray data show that CYP79B2 and known auxin responsive genes are among the most upregulated genes after ethanol treatment.

277 MEKK1 Negatively Regulates Tissue Specific and Temperature Dependent Cell Death in Arabidopsis

Kazuya Ichimura[1, 2], Catarina Casais[1], Scott Peck[1, 3], Kazuo Shinozaki[2], Ken Shirasu[1, 2]

[1]The Sainsbury Laboratory, John Innes Centre, Norwich, UK., [2]RIKEN Plant Science Center, Yokohama, Japan., [3]Department of Biochemistry, University of Missouri Columbia, MO

Innate immunity signaling pathways in plants are regulated by mitogen-activated protein kinase (MAPK) cascades. Using protoplast transient expression system, Arabidopsis MEKK1, a MAPK kinase kinase (MAPKKK) has been proposed as a positive regulator of innate immunity signaling in the upstream of the MKK4, MKK5 (MAPKKs) - MPK3, MPK6 (MAPKs) pathway (1). In addition, yeast two-hybrid and complementation analyses and protoplast transient expression experiment suggested that MEKK1 has a role in the upstream of the MKK1, MKK2 (MAPKKs) – MPK4 pathway (2, 3, 4). Flg22 treatment activates MPK3, MPK4, and MPK6 (1, 5). However, genetic evidence of MEKK1 involvement in flg22-triggered MAPK activation has not been demonstrated.

We show that loss of MEKK1 results in temperature-sensitive and tissue-specific cell death and H_2O_2 accumulation that are partly dependent on both RAR1, a key component in resistance (R) protein function, and SID2, an isochorismate synthase required for salicylic acid production upon pathogen infection. MEKK1 is not required for flg22-triggered activation of MPK3 and MPK6. Instead, MEKK1 is essential for activation of MPK4, which is a negative regulator of defense responses (6). These data suggest that MEKK1 plays an important role in negative control of defense responses.

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278 Application of Laser Microdissection to the study of a powdery mildew-Arabidopsis pathosystem

Noriko Inada[1], Mary Wildermuth[2]

[1]Nara Institute of Science and Technology, [2]University of California, Berkeley

The powdery mildew Golovinomyces orontii (formerly named Erysiphe orontii) is an obligate biotrophic fungus that infects epidermal cells of Arabidopsis thaliana leaves. When powdery mildew conidia are inoculated on a leaf surface, conidial germination occurs at 2 hrs after inoculation (hpi), followed by appresorial formation and penetration at 5 hpi, and haustorial formation at 24 hpi. Later, fungi grow mycelium on the leaf surface and develop conidiophores to produce more conidia. Most research elucidating the powdery mildew-Arabidopsis interaction has focused on later stages of infection when powdery mildew is visible to the naked eye (~5-7 dpi) due to the time-intensive nature of microscopic evaluation and lack of markers for early infection stages. In addition, conventional whole leaf analysis does not differentiate events in infected cells from those of neighboring uninfected cells. With this in mind, we are employing Laser Microdissection (LMD) to analyze populations of infected epidermal cells and uninfected epidermal and mesophyll cells separately. This powerful method has been utilized for animal research, yet is still new to plant biology. As the first step, we optimized fixation and paraffin embedding methods suitable for fragile mature Arabidopsis leaves. High quality histology as well as RNA were obtained from paraffin sections prepared by the microwave method. RT-PCR analysis clearly exhibited the specificity of laser dissected cells. We then optimized method for RNA extraction and amplification for the use with Affymetrix Arabidopsis GeneChip. We are currently in the process of microarray analysis for infected and uninfected cells at 5 dpi. The temporally and spatially resolved global expression data generated through this work will facilitate detailed mechanistic understanding of the powdery mildew-Arabidopsis interaction and derivation of the regulatory circuitry associated with this interaction.

279 Mechanisms controlling coordinated transcriptional reprogramming of defense genes in Arabidopsis

Colleen Knoth, Thomas Girke, Thomas Eulgem

ChemGen IGERT Program, Center for Plant Cell Biology (CEPCEB) & Department of Botany and Plant Sciences, University of California, Riverside, USA

We use interactions of *Arabidopsis thaliana* (Arabidopsis) and *Hyaloperonospora parasitica (Hp*, Peronospora) to study transcriptional reprogramming during plant-pathogen interactions. Specific Arabidopsis disease resistance *(R)* genes recognize distinct *Hp* isolates and trigger signaling cascades leading to resistance. Using microarrays, Arabidopsis genes were identified that exhibit a strong and coordinated **L**ate/sustained **U**p-regulation in **R**esponse to **P**eronospora recognition (*LURPs*). T-DNA mutations in individual *LURP* genes cause partial defects in resistance to *Hp*, suggesting their concerted activity and coordinated up-regulation is required for full resistance. We initiated multiple approaches to uncover regulatory mechanisms coordinating *LURP* expression. We have identified *LURP* gene *AtWRKY70*, encoding a WRKY transcription factor, as one key control point in this immune response. In addition, using reporter gene assays we have isolated promoter regions of two representative *LURP* genes containing several novel candidate *cis*-elements that may contribute to their co-regulation. Further differentiation of these putative *cis*-elements and identification of corresponding transcription factors will provide insight into the mechanisms controlling this important regulatory step. In addition, we are conducting chemical genomics screens to find compounds that perturb the expression of *LURP-promoter::GUS* fusions in the absence of *Hp*. Follow-up screens for mutants that are insensitive or hypersensitive to the identified small molecules will be used to identify protein targets. We anticipate the identification of chemicals that activate parts of the *Hp* defense pathway by interference with R proteins or other known or novel pathway components. These elicitors will be invaluable tools for the dissection of mechanisms controlling the plant defense network and may lead to the development of agrochemicals with the ability to utilize the gene-for-gene resistance program inherent to plants. (Supported by NSF-IGERT grant DGE 0504249 and NSF grant 0449439).

280 Lipids as Signaling Molecules in Plant Defense

Kartikeya Krothapalli[1], Ratnesh Chaturvedi[1], Ashis Nandi[2], Ruth Welti[1], Jyoti Shah[1]

[1]Kansas State University, [2]Jawaharlal Nehru University

Systemic acquired resistance is an inducible defense mechanism that is activated in the naive organs of a plant that had previously been inoculated with a necrogenic pathogen. SAR confers enhanced resistance against a variety of pathogens. The activation of SAR requires the translocation through the phloem of an unknown signal from the pathogen-inoculated organ to the organs that will exhibit SAR. Our previous studies in Arabidopsis had suggested the involvement of a lipid in the activation of systemic acquired resistance (SAR) (Nandi et al. 2004). In our attempts to identify the lipid(s) that is important for SAR, we have integrated the power of genetics with lipidomics, a highly sensitive technology to characterize lipids in biological system. Our recent studies with the Arabidopsis sfd2, fad7 and mgd1 mutants, along with our past studies of the ssi2 and sfd1 mutant suggest that chloroplastic lipids have an important role in SAR. In particular, these studies suggest the involvement of a chloroplast-derived galactolipid or a product thereof in the activation of SAR. We will present our progress on understanding the role of lipids in SAR.

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281 *Arabidopsis thaliana* Defense Response Triggered by *Brevicoryne brassicae* Attack Has Different Intensity in Ecotypes with Diverse Glucosinolate Profiles

Anna Kusnierczyk[1], Per Winge[1], Herman Midelfart[1], John Rossiter[2], Scott Armbruster[1], Atle Bones[1]

[1]Department of Biology, The Norwegian University of Science and Technology, Realfagbygget, 7491Trondheim, NO, [2]Department of Biology, Imperial College at Wye, University of London, Wye, Kent TN25 5AH, UK

Phloem piercing-sucking insects are an important factor that limits crop plants production. Aphids, despite sophisticated feeding strategy minimizing cell damage, activate responses broadly similar to those triggered by pathogen attack, chewing herbivore and wounding. Defense strategies of most plants involve signaling molecules such as jasmonic acid (JA), salicylic acid (SA) and ethylene (ET), along with glucosinolates and their hydrolysis products in cruciferous species. Pests which specialized to feed on *Brassicaceae* have evolved various mechanisms to protect themselves against toxic products of glucosinolate hydrolysis. Cabbage aphid, *Brevicoryne brassicae,* produces an enzyme similar to plant myrosinase, which is able to hydrolyze plant glucosinolates. We used oligonucleotide microarrays to study the changes in transcriptional profile of plants infested with *B. brassicae*. Three *Arabidopsis thaliana* ecotypes differing in predominant products of glucosinolate hydrolysis were chosen: Wassilewskija (Ws), Cape Verde Islands (Cvi), and Landsberg erecta (Ler), which produce mainly isothiocyanates, epithionitriles and nitriles, respectively. In all three ecotypes, the major pathways involved in plant defense were up-regulated upon aphid attack, but the expression level of genes involved in these pathways varied. The JA synthesis pathway was induced highest in Cvi, while the indolic glucosinolate synthesis pathway was strongest up-regulated in Ler. In contrast, two genes coding for plant myrosinases: thioglucoside glucohydrolase 1 and 2 (TGG1 and TGG2) were down-regulated.

282 Salicylic Acid-Mediated Innate Immunity in *Arabidopsis* is Regulated by SIZ1 SUMO E3 ligase

Jiyoung Lee[1], Jaesung Nam[2], Kenji Miura[3], Jingbo Jin[3], Chan Yul Yoo[3], Paul M. Hasegawa[3], Dae-Jin Yun[1]

[1]Gyeongsang National University, [2]DongA University, Pusan, South of Korea, [3]Purdue University, IN, USA

Reversible post-translational modification of proteins by *s*mall *u*biquitin-related *mo*difier (SUMO) protein is involved in many important cellular processes in yeasts and animals. However, little is known about the function of sumoylation in plants. In this study, we show that the *SIZ1* gene encoding an Arabidopsis SUMO E3 ligase regulates innate immunity. siz1 plants exhibit constitutive systemic acquired resistance (SAR) that is characterized by elevated accumulation of salicylic acid (SA), increased resistance to the bacterial pathogen Pseudomonas syringae pv. tomato (*Pst*) DC3000 and constitutive pathogenesis-related (PR) gene expression. In *siz1* plants expressing SA hydroxylase *nahG* (*siz1-2 nahG*), disease resistance is linked to elevated SA levels. In addition, levels of *PAD4*, *EDS1*, *EDS5* and *SID2* transcripts were induced strongly, whereas expression of *NDR1* or *NPR1* was similar to wild-type. The effect of *siz1* on SA signaling was studied using the double mutants *siz1*npr1, siz1pad4 and siz1ndr1. SIZ1 and PAD4 interact epistatically to regulate PR expression and disease resistance. Consistent with these observations, *siz1* plants exhibited enhanced resistance to *Pst* DC3000 expressing *avrRps4*, a bacterial avirulence determinant that responds to the EDS1/PAD4-dependent TIR-NBS type R gene. In contrast, *siz1* plants did not demonstrate resistance to *Pst* DC3000 expressing *avrRpm1*, a bacterial avirulence determinant that responds to the NDR1-dependent CC-NBS type R gene. Jasmonic acid (JA)-induced *PDF1.2* expression and susceptibility to Botrytis cinerea were unaltered in siz1 plants. Taken together, our results suggest that SIZ1 regulates PAD4-mediated SA signaling, which in turn confers innate immunity in Arabidopsis.

283 *GLZ1*, a member of family 8 glycosyltransferase, may play a role in programmed cell death

Tsai-Chi Li[1], *John Price*[1], *Jyan-Chyun Jang*[1, 2]

[1]Department of Horticulture and Crop Science, The Ohio State University, Columbus, (OH), USA,
[2]Department of Plant Cellular and Molecular Biology, The Ohio State University, Columbus, (OH), USA

Programmed cell death (PCD) is a regulated process that is critical for plant survival and development. Localized cell death can block the spread of the infection during pathogen attack. For certain pathogens, an infection can trigger oxidative burst and generate reactive oxygen intermediates (ROIs). The ROIs can in turn induce salicylic acid (SA) accumulation and induce the PCD. The Arabidopsis dwarf mutant glz1 was initially identified as having defects in development as well as sugar accumulation and translocation (Plant Cell Physiol. 45:1453-1460). GLZ1 encodes a glycosyltransferase with a sequence highly homologous to Avr9/Cf-9 Rapidly Elicited (ACRE231) protein in tobacco. The *glz1* mutants seem to be tolerant to spontaneous powdery mildew infections, where macroscopic cell death lesions are not apparent on infected plants. To understand the mechanisms underlying this abnormal response, we conducted artificial inoculations using fungal pathogens under a controlled environment. Unlike the typical hypersensitive response seen in wild type plants, in which a few large lesions were found, hundreds of small lesions were formed on glz1 plants. However, these micro-lesions could neither expand nor effectively prevent the pathogen from spreading to surrounding healthy tissues. Interestingly, while SA application on wild-type leaves can cause high levels of ROIs accumulation and cell death, glz1 plants were unaffected by the SA treatment. However, glz1 plants were not completely insensitive to SA because SA-induced PR1 and PR5 expression appeared to be normal. We hypothesize that the lack of SA-induced cell death may be due to defects in ROI accumulation and signal transduction. This notion is supported by evidence indicating that low levels of H_2O_2 accumulation and compromised cell death (ACD6) gene induction occur when glz1 plants are treated with INA or SA. The glz1 defect appears to have a profound effect on defense and cell death response, because microarray analyses revealed a trend of down regulation of ACD6, MEK1, RIN4, WAK1, and WRKY70 in the absence of pathogen infection.

284 The mRNA of a putative Proline-rich Protein is down-regulated during the wounding response in Arabidopsis

Chenggang Liu, Mona Mehdy

The University of Austin at Austin

Proline-rich Proteins (PRPs), known as glycosylated cell wall structural proteins, have been broadly implicated in various plant developmental stages and the plant defense response. Some PRPs, which have high tyrosine content, become insolubilized in the cell wall by wounding or fungal elicitor treatment. In contrast, low tyrosine content PRPs such as PvPRP1 in French bean are believed to be not involved in this cell wall strengthening process. Previously, our lab had shown PvPRP1 mRNA was down-regulated through mRNA degradation upon wounding and fungal elicitor treatment in French bean. Here, we report an Arabidopsis mRNA encoding a putative low tyrosine PRP (AtPRP5) that is down-regulated by wounding, MeJA and ABA treatment. The mRNA level of AtPRP5 decreased to about 30% of that in untreated plants in 8 hr. The maximum half-life of AtPRP5 mRNA was estimated to be about 4 hr. Unlike PvPRP1, nuclear run-on assay showed that the down-regulation of AtPRP5 mRNA upon MeJA treatment was mainly transcriptional. The luciferase reporter gene fused with the 3' UTR of AtPRP5 mRNA was stably transferred into plants. The results indicated that the 3'UTR was not involved in AtPRP5 mRNA stability regulation. Based on Northern blots, roots and inflorescence have the most abundant mRNA expression level; stem and root have much lower expression level. Transgenic plants with a promoter-GUS fusion indicated that AtPRP5 gene was transcriptionally active in the vascular bundle region throughout the plants. The mutant plants with a transposon insertion in AtPRP5 gene did not show any difference from wild type plants under normal growth conditions. However, the seeds of this mutant showed significant insensitivity to ABA inhibition during seed germination. Together, these results suggest that AtPRP5 plays a specific role in plant development.

285 Screening for Suppressors of Arabidopsis acd11

Frederikke Malinovsky[1], Peter Brodersen[2], Daniel Hofius[1], Lea McKinney[1], Nikolaj H Petersen[1], Morten Petersen[1], Berthe Fiil[1], John Mundy[1]

[1]institue of molecular biology, university of copenhagen, [2]Institut de Biologie Moleculaire des Plantes du CNRS

Despite attempts to link plant cell death to animal apoptosis, comparative analyses of the genetic determinants of programmed cell death (PCD) in plants and animals have yet to identify conserved gene functions. Loss of function of the Arabidopsis gene Accelerated Cell Death 11 (ACD11; Brodersen et al. 2002 Genes & Develop. 16, 490; Brodersen et al. 2005 Plant Physiol. 138, 1037) activates vegetative cell death and disease resistance responses dependent upon the hormone salicylate (SA). Double mutant analysis showed that of 12 mutants affected in defense-associated PCD (eds1, pad4, eds5, sid2, npr1, rar1, pbs1, pbs3, ein2, etr1, jar1, ndr1), only two (eds1 and pad4) suppress acd11 PCD in the presence of the SA analog BTH. To further understand ACD11 function, we are searching for PCD regulators in a large-scale acd11 suppressor screen. More specifically, acd11 homozygotes expressing the bacterial SA hydroxylase nahG were mutagenized and plants surviving BTH treatment isolated as putative suppressors. This identified a number of recessive and dominant suppressors of acd11. Recent work has sorted the recessive mutants fall into fifteen complementation groups. Sequencing has confirmed that two of these groups are allelic to eds1 validating the utility of the screen.

286 Analysis of a nonpathogenic strain of Pseudomonas syringae for use in host range and pathogenicity studies on Arabidopsis thaliana and tomato

Toni Mohr[1], Ryan Anderson[1], Peter Bowerman[1], Nina Long[1], Leiya Williams[1], Joanna Jelenska[2], Jose Castillo[2], Jean Greenberg[2], Boris Vinatzer[1]

[1]Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Fralin Biotechnology Center, West Campus Drive, Blacksburg, VA 24061-0346, USA, [2]Department of Molecular Genetics and Cell Biology, The University of Chicago, 1103 East 57th Street, EBC410, Chicago, IL 60637, USA

The bacterial plant pathogen Pseudomonas syringae encompasses over 50 pathovars that collectively cause disease on hundreds of plant species. However, any particular strain is limited to one or a few plant hosts. This host range is determined by numerous bacterial effector proteins, which are injected into a plant cell by a Type III secretion system (TTSS). We are examining how TTSS effector proteins affect the host range of Arabidopsis and tomato pathogens. Here we characterize a nonpathogenic strain, Psy508, originally isolated as a biocontrol strain against the apple scab fungus. Although closely related to the bean pathogen PsyB728a, Psy508 is unable to cause disease on any tested plant species. Interestingly, Psy508 does not appear to have a functional TTSS, which is essential for pathogenicity. The typical P. syringae hrp/hrc cluster with flanking effector loci coding for the TTSS machinery and effectors is absent in this strain, and has been replaced by a bacteriophage sequence. Additionally, Psy508 appears to be missing most effector genes elsewhere in the genome. We also sequenced three genomic islands that contain effectors in PsyB728a; these islands are either rearranged, or lack effector genes in Psy508. Psy508 may thus be a useful strain in which to study the effects of individual effectors, or combinations of effectors, isolated from other strains with different host ranges. We are currently cloning the TTSS, without the flanking effector loci, from PsyB728a. By adding this TTSS and individual effectors to Psy508, we plan to analyze the role of effector proteins in determining the host range of Pseudomonas syringae.

287 Arabidopsis as a Model System to Study Plant Defense Against Fusarium graminearum, the Causative Agent of Scab in Wheat and Barley

Vamsi Nalam, Ragiba Makandar, Darcy Maier, Harold Trick, Jyoti Shah
Kansas State University

Fusarium head blight (FHB) is a common and devastating disease of wheat and barley around the world. In the US, yield losses due to FHB in some years have reached $1 billion. Fusarium graminearum Schwabe is the principal causal agent of FHB. Unlike many other diseases, monogenic gene-for-gene resistance to FHB has not been identified and the mechanism(s) involved in signaling and activation of plant defense against F. graminearum are poorly understood. A host-fungus system consisting of Arabidopsis-F. graminearum provides an excellent model system to study and rapidly identify genes involved in signaling and activation of plant defenses. We have observed that constitutive overexpression of the Arabidopsis NPR1 (AtNPR1) gene confers enhanced resistance against F. graminearum in Arabidopsis and wheat (Makandar et al. 2006), suggesting conservation of defense mechanisms against this pathogen in Arabidopsis and wheat. Furthermore, SA and BTH application also enhance resistance against this fungus. Our results indicate that constitutive expression of AtNPR1 primes wheat defenses to respond faster to SA and the fungus. Our studies in Arabidopsis have identified other components of host defense against F. graminearum. In addition, we have identified an Arabidopsis gene, which contributes to F. graminearum virulence. Progress on this work will be presented.

Makandar, R., Essig, J. S., Schapaugh, M. A., Trick, H. N. and Shah, J. 2006. Genetically engineered resistance to Fusarium head blight in wheat by expression of Arabidopsis NPR1. Mol. Plant-Microbe Interact. 19:123-129.

288 Plant Growth and Pathogen-Related Response Are Regulated via Glutathionylation of a Single Protein

Ken'ichi Ogawa[1, 2], Masayoshi Matsumoto[1, 2], Hatano-Iwasaki Aya[1, 2], Kenji Henmi[1, 2]
[1]RIBS OKAYAMA (Res. Inst. Biol. Sci. Okayama), [2]CREST, JST

We have recently been reporting that glutathione (GSH), an abundant antioxidant, regulates many physiological events in plants [Ogawa (2005) Antioxid. Redox Signal. 7: 973-981]. Since most of the events that are governed by GSH cannot be controlled by other thiols, we focused on and studied the physiological function of proteins undergoing glutathionylation (covalent biding of the GSH moiety through the disulfide bride). Here I will present a talk showing that one of proteins undergoing glutathionylation in chloroplasts plays a key role in plant growth and pathogen-related response and that glutathionylation determines the function of the protein. Considering that GSH synthesis is strongly dependent on photosynthesis and that plant growth and pathogen-related response are regulated by GSH, it can be concluded that plant growth and pathogen-related response are regulated by photosynthesis via glutathionylation of the single protein.

289 Resistance to *Xanthomonas campestris* pv. *campestris* in *Arabidopsis thaliana*

Laure Perchepied, Raimana Louette, Damien Meyer, Kroj Thomas, Dominique Roby
LIPM CNRS/INRA Toulouse

Xanthomonas campestris pv. *campestris (Xcc)* is a model pathogen which causes black rot disease on crucifers. For studying the interaction of *Xcc* with the model plant *Arabidopsis thaliana,* a wound inoculation test was developed in the laboratory (Meyer *et al*., 2005). The genetic determinism for resistance to *Xcc* in *Arabidopsis thaliana* is not yet understood. To get an insight into this process, we first tested Arabidopsis mutants impaired in resistance to different pathogens in order to determine if known signalling components of resistance are involved in resistance to *Xcc*. While mutants impaired in specific resistance to other pathogens do not seem to be affected in resistance to *Xcc,* different *pad* and *eds* mutants which possess reduced basal resistance, such as *pad1,* were found to be susceptible to *Xcc*.

Then, we started to investigate the genetic bases of resistance to *Xcc* in Arabidopsis without *a priori*. We analysed various Arabidopsis accessions and identified high natural variation for resistance to *Xcc*. The accession Columbia 5 (Col-5) is for example resistant to *Xcc* whereas Kashmir (Kas) is susceptible. In order to identify the loci that confer resistance, we analysed a recombinant inbred (RILs) population (110 lines) derived from a cross between Col-5 and Kas. We performed four independent tests in a complete randomized design. The RILs distributions showed a continuous variation of resistance between the resistant accession Col-5 and the susceptible accession Kas, suggesting a polygenic control of resistance. Quantitative trait loci (QTL) analyses were performed on the four independent tests and allowed the detection of one major and 3 minor QTLs. In order to characterize the locus corresponding to the major QTL, we used heterogeneous inbred families (HIF) from the RIL population. These HIFs allowed us to validate the effect of the major QTL. Fine mapping of this locus is now underway. With the development of numerous markers, we could reduce the confidence interval of the QTL to 2.3Mb.

Meyer et al., Mol Plant Pathol (2005) 6 (3), 327-333.

290 Functional characterization of the ACD11 protein

Nikolaj H. Petersen[1], Peter Brodersen[2], Margerita Malakova[3], Daniel Hofius[1], Lea Mckinney[1], Helen Pike[3], Rhoderick Brown[3], John Mundy[1]
[1]Institute of Molecular Biology, University of Copenhagen, Denmark, [2]Institut de Biologie Moleculaire des Plantes CNRS, Strasbourg, France, [3]Hormel Institute, University of Minnesota, Austin, Minnesota, USA

Knockout of the *Arabidopsis Accelerated Cell Death 11* gene leads to a defense-related, constitutive programmed cell death (PCD) reaction and the recessive *acd11* mutant is non-viable. The ACD11 protein shows homology to mammalian glycolipid transfer protein (GLTP), that transfer glycosphingolipids between membranes. However, ACD11 was shown to transfer the sphingobase sphingosine, a different substrate than GLTP. Sphingolipids are important components of plasma membranes, but spingobases such as ceramide and sphingosine have also been shown to have signaling properties. Mutational analysis of GLTP revealed amino acids that are required for transfer activity, and that are conserved in ACD11. We are currently testing whether corresponding site-directed mutant ACD11 forms can complement the *acd11* phenotype. Preliminary data suggest that at least two of these mutants complement, but that they are affected in aspects of plant defence. We are also using a previously described FRET based lipid transfer assay to further elucidate the substrate specificity of ACD11, as well as testing the activity of mutant forms of ACD11.

291 The NIMIN-NPR1 Connection: A Molecular Switch that Controls the Expression of PR Genes

Ursula Pfitzner, Sylvia Zwicker, Vlatka Stos
Universitaet Hohenheim

NPR1 (also known as NIM1) is a positive key regulator of systemic acquired resistance (SAR) in Arabidopsis. Upon salicylic acid (SA) induction, oligomeric NPR1 localized in the cytoplasm is mobilized (Mou et al., 2003). NPR1 monomers enter the nucleus, and transcription of pathogenesis-related (PR) genes starts (Kinkema et al., 2000). NPR1 interacts with two classes of proteins, TGA transcription factors (Zhang et al., 1999) and a group of novel proteins, termed NIMIN proteins (NIMIN = NIM1 interacting protein; Weigel et al., 2001, 2005). In Arabidopsis, NIMIN1, NIMIN2, and NIMIN3 constitute a small gene family of structurally related, yet distinct members. NIMIN-type genes are found throughout the plant kingdom. G8-1, for example, a tobacco NIMIN homolog, has been reported to be rapidly and sensitively induced by SA (Horvath et al., 1998). Likewise, both the Arabidopsis NIMIN1 and NIMIN2 promoters are responsive to SA (Glocova et al., 2005).

To unravel their biological function, we have started to characterize the tobacco NIMIN group. cDNA clones were isolated encoding G8-1 (from here on called NtNIMIN2a) and two novel family members. The NtNIMIN proteins are structurally related to each other exhibiting the highest similarity to AtNIMIN2. For functional studies, we have expressed a 35S::NtNIMIN2a chimeric gene and a NtNIMIN2a RNAi construct in tobacco. While overexpression of NtNIMIN2a inhibited PR-1 proteins, suppression of NtNIMIN2 transcripts led to enhanced PR-1 protein accumulation. In both cases, the effects of altered NtNIMIN2 transcript levels became evident foremost at the onset of SAR, indicating that NtNIMIN2 proteins repress PR-1 genes transiently. We suggest that NIMIN proteins are positive regulators of the SAR response that supervise PR gene expression through a novel mechanism termed signal-mediated pre-transcriptional control (SIMPC). SIMPC may provide the molecular basis for the phenomenon of priming. In Arabidopsis, SIMPC has adopted to the different lifestyle of the plant. Consequently, control of PR gene expression is mediated by distinct types of NIMIN proteins, NIMIN1, NIMIN2, and NIMIN3.

292 Arabidopsis thaliana; A Naive Species To Study Plant/Virus Interactions

Veronique Decroocq, Micheline Lansac, Hien Le, Ophelie Sicard, Thierry Candresse, Olivier Le Gall, Frederic Revers
UMR GDPP, INRA Bordeaux-Universite Bordeaux2, BP81, 33883 Villenave d'Ornon Cedex, France. http://www.bordeaux.inra.fr/ipv/

The virus cycle of infection in plant is a complex process that includes the expression of the viral genome, suppression of host defences, virus replication, cell-to-cell movement via plasmodesmata and long distance movement through the vascular system. This multi-step process requires interactions between factors from both the host and the virus. Unfortunately until now, very few host factors have been identified.

In the aim to identify such factors, *Arabidopsis thaliana* accessions were challenged with isolates of two potyviruses, *Plum pox virus* (PPV) and *Lettuce mosaic virus* (LMV), which naturally infect *Prunus* and *Lactuca* species respectively. A high level of variability was observed both in the behaviour of LMV(1) and PPV(2) isolates and in that of the various accessions tested, suggesting a high level of diversity of interactions within these naive Arabidopsis/potyvirus pathosystems. Phenotypes ranged from complete systemic invasion, accompanied or not by symptoms, to the inability of the virus to mount a productive replication in the initially inoculated cells. Several genetic factors playing a key role in the success of infection were identified and their clonings are in progress:

(i) the restriction of long-distance movement of LMV and PPV in the Col accession requires the products of the RTM genes, up to now believed to be specific to another potyvirus, TEV.

(ii) the restrictions of long-distance movement of LMV and PPV in accession Cvi are controlled by the *rlm1* and *rpv1* recessive resistance genes respectively, which were mapped in distinct genetic intervals on chromosome 1.

(iii) the symptoms induced by one PPV isolate in accession Ler is controlled by several QTLs mapped in different parts of the Arabidopsis genome.

To extend this study, a core-collection (24 Arabidopsis accessions) representing 96% of the intra-specific genetic diversity has been screened by a small panel of PPV and LMV isolates covering the genetic diversity of each virus. This work allowed to identify new resistance phenotypes as well as situations with generalized symptomatic infections. The involvement of the corresponding new host genes in the Arabidopsis/potyvirus interactions will be further analysed.

(1) Revers F. ***et al***., 2003. MPMI 16, 608-616.
(2) Decroocq V. ***et al***., 2006.MPMI 19, 541-549**.**

293 A Role for a Flavin-Containing Monooxygenase in Resistance against Microbial Pathogens in Arabidopsis

Martina Koch[1], Sonja Vorwerk[2], Clarissa Masur[1], Gholamreza Sharifi-Sirchi[1], Nicoletta Olivieri[1], Nikolaus Schlaich[1]
[1]RWTH Aachen University, Institut Bio III (Pflanzenphysiologie), Aachen, Germany, [2]Carnegie Institution of Washington, Department of Plant Biology, Stanford, CA, USA

Using activation-tagging in the Arabidopsis Col-0 rps2-101C background we identified a mutant (SRS1-D) that showed virtually no symptoms after inoculation with virulent Pseudomonas syringae pv. tomato DC3000 bacteria. The dominant, gain-of-function phenotype of the SRS1-D mutant is due to over-expression of a class 3 flavin-containing monooxygenase (FMO). We recapitulated the SRS1-D mutant phenotype in independent transgenic Col-0 lines over-expressing the FMO cDNA under control of the 35S CaMV promoter. The increased basal resistance observed in the SRS1-D mutant was also effective against the taxonomically unrelated downy mildew-causing pathogen Hyaloperonospora parasitica. By investigating the progeny from crosses of SRS1-D mutant with the NahG transgenic line we showed that the enhanced basal resistance phenotype was dependent on the accumulation of salicylic acid. SRS1-D plants showed wild-type resistant reactions after inoculation with avirulent bacteria, indicating that the R-gene-mediated defence physiology was not compromised by FMO over-expression. Transcripts of the class 3 FMO gene accumulated within 6 hours after inoculation of wt Col-0 plants with avirulent Pst+avrRpt2 cells. Moreover, a T-DNA insertion into the SRS1 gene results in enhanced susceptibility to virulent Pseudomonas and Hyaloperonospora parasitica, suggesting that expression of the FMO gene is a hitherto undescribed component of the plant's resistance repertoire. We discuss the possibility that the FMO may participate in the detoxification of virulence factors produced by pathogens.

294 Transcriptomic analysis of potyvirus-infected plants: Comparative study between *Arabidopsis thaliana* and tomato

Xao Yan Feng[1], Maud Rivard[1], Johann Petit[2], Ludivine Taconnat[3], Christophe Rothan[2], Jean-Pierre Renou[3], Valerie Schurdi-Levraud[1, 4], Olivier Le Gall[1], Carole Caranta[5], Frederic Revers[1]
[1]UMR GDPP, INRA Bordeaux-Universite Bordeaux 2, BP81, 33883 Villenave d'Ornon cedex, France, [2]UMR PBV, INRA Bordeaux-Universite Bordeaux 1 et 2, Villenave d'Ornon, France, [3]URGV, INRA Evry, France , [4]AGRO Montpellier, France, [5]UGAFL, INRA, Montfavet, France

With the aim to identify and compare host genes of two species differentially regulated in the presence of an infecting virus, *Arabidopsis thaliana* Landsberg erecta (Ler) accession and tomato cv. Microtom were challenged with Tobacco etch potyvirus (TEV) which is able to infect both species. Microarrays slides from Cornell University for tomato and from the CATMA project for *Arabidopsis* were respectively hybridized with total RNA extracted from TEV infected and mock inoculated microtom plants or Ler plants 7 days post inoculation. The patterns of the differentially expressed genes were evaluated in inoculated and systemic leaves.

More than 1500 genes were found to be deregulated in TEV infected *Arabidopsis* and about 650 in tomato. In both species a ten-fold difference in deregulated genes were found between inoculated leaves and systemic leaves. Few commun homologous genes have been found to be differentially expressed in both species.

295 A Search for Transcriptional Regulators of Disease Resistance

Natalie Spivey, Dong Wang, Xinnian Dong
Duke University

In response to pathogen attack, the plant activates several different signaling pathways and initiates changes in gene expression. While some of the transcriptional changes are well characterized and used as markers for the defense response, questions remain about the regulation of the transcriptional activation and repression events induced by infection. Using microarray technology, we sought to characterize the changes in gene expression that resulted from infection with the bacterial pathogen Pseudomonas syringae. At five different time points, we infected half of an Arabidopsis leaf with either virulent P. syringae or avirulent P. syringae/avrRpt2. The uninfected half of the leaf was then collected and prepared for microarray analysis using the Affymetrix GeneChip Arabidopsis ATH1 Genome Array. Using this approach, we were able to compare each gene's expression pattern over time. We are currently using these data to search for transcription factors that regulate the expression changes that result from pathogen infection. We are also looking for potential cis-acting regulatory elements in the promoter regions of genes whose expression pattern is highly correlated.

296 Arabidopsis Virulence of Xanthomonas campestris pv. campestris Mutants Defective in Secretion Systems

Wenxian Sun, Muthu Venkateshwaran, Andrew Bent
Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, USA

The bacterial plant pathogen *Xanthomonas campestris* pv. *campestris* causes disease in crucifer plants including *Arabidopsis thaliana*. We recently showed that *Xcc* strains that make a flagellin detectable by the Arabidopsis FLS2 RLK nevertheless caused similar disease on FLS2+ and fls2-/fls2- plants (Sun et al., 2006. Plant Cell 18:491). In *Pseudomonas syringae* pv. *tomato* DC3000, both type III secretion and twin-arginine translocation systems were shown to contribute to virulence and fitness. *Xanthomonas campestris* pv. *vesicatoria* virulence was attenuated when the bacteria were defective in pilus secretion. Here, we explored the contribution of the type III secretion system (TTSS) and twin-arginine translocation (TAT) system to *Xcc* virulence and fitness using TTSS- and TAT-defective mutants. *HrcC* and *hrpE* genes encode central key components of the type III secretion apparatus. *HrcC* and *hrpE* gene deletions caused different phenotypes in different *Xcc* strains. *Xcc* B186 strain virulence on Arabidopsis was greatly attenuated when TTSS was knocked out while *Xcc* B305 *ΔhrcC* strains had similar virulence ability to the isogenic wildtype B305 strain based on bacterial numbers in infected plants after vacuum infiltration or hydathode inoculation. *Xcc* mutants defective in TAT system exhibited similar pleiotropic phenotypes to a *Pst* DC3000 *tatC* knockout strain, such as hypersensitivity to copper. However, unlike *Pst* DC3000, the twin-arginine translocation system played a trivial role in *Xcc* virulence, as tested using two different inoculation approaches. Based on these data, it is speculated that other secretion systems are likely to exist in *Xcc* bacteria that contribute significantly to virulence. Mutants defective in other secretion systems are being made to test our hypotheses. Interestingly, TTSS mutants will also be used to test the function of TTSS virulence effectors in the suppression of PAMP-induced plant basal defenses.

297 Rapid and economic mapping of regulatory loci in complex traits using gene expression profiling

Remco Van Poecke, Masanao Sato, Lisa Lenarz-Wyatt, Fumiaki Katagiri
University of Minnesota

Genetic variation is a useful tool to dissect complex traits, such as inducible defense against pathogens in plants. An excellent source of genetic variation can be found in naturally occurring populations. However, use of such natural variation is hampered by the quantitative nature of complex traits. Thus, dissection of complex traits using natural variation commonly involved tedious genetic crossing schemes and genotypic analyses, such as creation, phenotyping, and genotyping of recombinant inbred lines. We are currently developing a rapid and economical method to detect and map regulatory loci involved in complex traits. The concept of this method is to finely dissect the phenotype for a complex trait using expression profiles and to identify parts of phenotype (i.e., expression of some genes) that segregate in a Mendelian manner and that are controlled in trans. We initiated characterization of Arabidopsis accessions in response to infection of an avirulent bacterial strain by conventional phenotyping and expression profiling. To reduce the cost of expression profiling, we are using a dedicated small-scale DNA microarray. We will report that the dedicated microarray is an excellent tool to study natural variation in Arabidopsis and that results of the phenotypic and expression profile characterization of the parental accessions are well correlated.

This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2004-35301-14525.

298 Arabidopsis Bax-Inhibitor 1 Functions As An Attenuator Of Biotic And Abiotic Types of Cell Death

Naohide Watanabe, Eric Lam
Biotech Center, Rutgers The State University of New Jersey

Programmed cell death (PCD) is a common process in eukaryotes during development and in response to pathogens and stress signals. Bax inihibitor-1 (BI-1) is a small protein of 25-27 kDa with 6 or 7 predicted transmembrane domains and is mainly localized in the membrane of the endoplasmic reticulum (ER). BI-1 is proposed to be a cell death suppressor that is conserved in both animals and plants, but the physiological importance of BI-1 and the impact of its loss of function in plants are still unclear. In this study, we identified and characterized two independent Arabidopsis mutants with a T-DNA insertion in the AtBI1 gene. The phenotype of atbi1-1 and atbi1-2, with a C-terminal missense mutation and a gene knockout, respectively, was indistinguishable from wild-type plants under normal growth conditions. However, these two mutants exhibit accelerated progression of cell death upon infiltration of leaf tissues with a PCD-inducing fungal toxin fumonisin B1 (FB1) and increased sensitivity to heat shock-induced cell death. Under these conditions, expression of AtBI1 mRNA was up-regulated in wild-type leaves prior to the activation of cell death, suggesting that increase of AtBI1 expression is important for basal suppression of cell death progression. Overexpression of AtBI1 in the two homozygous mutant backgrounds rescued the accelerated cell death phenotypes. In addition, we found that AtBI1 expression is enhanced under the ER stress conditions, suggesting that AtBI1 could modulate ER stress-induced cell death. Together, our results provide direct genetic evidence for a role of BI-1 as an attenuator for cell death progression triggered by both biotic and abiotic types of cell death signals in Arabidopsis.

N. Watanabe & E. Lam (2006) Arabidopsis Bax Inhibitor-1 functions as an attenuator of biotic and abiotic types of cell death. Plant Journal 45(6):884-894.

299 Identification of genes contributing to nonhost resistance of *Arabidopsis thaliana* against *Phytophthora infestans*

Lore Westphal[1], Joern Landtag[1], Mandy Birschwilks[1], Volker Lipka[2], Jan Dittgen[3], Paul Schulze-Lefert[3], Dierk Scheel[1], Sabine Rosahl[1]

[1]Leibniz Institute of Plant Biochemistry, D-06120 Halle, Germany, [2]ZMBP, University of Tuebingen, D-72076 Tuebingen, Germany, [3]Max-Planck Institute for Plant Breeding Research, D-50829 Koeln, Germany

Nonhost resistance is a characteristic feature of most interactions between plants and microorganisms, and it describes the resistance of a complete plant species against all members of a pathogen species. Colonization of a plant by a nonadapted pathogen is prevented by constitutive and preformed barriers or by the induction of multiple layers of defense responses upon recognition of the pathogen. One example for an active nonhost resistance is the resistance of *Arabidopsis thaliana* against the oomycete *Phytophthora infestans*, the causal agent of late blight disease on potato and tomato. In general, *P. infestans* spores germinate on Arabidopsis leaves and appressorium-like structures are formed. In most cases, penetration of the epidermal cell wall is averted by papillae formation. However, if the invasion attempt is successful, additional defense responses are activated, usually resulting in the death of the attacked epidermal cell. One of our approaches towards the genetic dissection of this phenomenon is a mutant screen based on the penetration resistance mutant *pen2*. *Pen2* encodes a glycosyl hydrolase, a component of an inducible preinvasion resistance mechanism that restricts entry of the nonadapted biotrophic ascomycetes *Blumeria graminis* f. sp. *hordei* and *Erysiphe pisi* as well as the hemibiotrophic oomycete *P. infestans* (Lipka et al. 2006, Science 310, 1180-1183). In comparison to wild type Arabidopsis, the *pen2* mutant shows an increase in dead epidermal cells after inoculation with *P. infestans* resulting in macroscopically visible necrosis. With the aim to impair additional resistance layers, *pen2* seeds were EMS-mutagenized, and approximately 70.000 M2-plants were scored with regard to their hypersensitive response phenotype after inoculation with *P. infestans*. So far, 32 mutants with enhanced cell death in comparison to *pen2* were isolated, and three mutations were roughly mapped on chromosome 1, 3 and 5, respectively.

300 Spatial and temporal analysis of host gene expression in response to infection by positive-strand RNA viruses

Chunling Yang[2], Rong Guo[2], Fei Jie[2], Dan Nettleton[2], Jiqing Peng[2], Tyrell Carr[2], Joanne Yeakley[1], Jian-Bing Fan[1], Steve Whitham[2]

[1]Illumina, Incorporated, San Diego, CA, USA, [2]Iowa State University, Ames, IA, USA

Systemic viral infections cause a variety of changes in plant gene expression in *Arabidopsis thaliana* that may be spatially and temporally regulated. To investigate this possibility, we used two complementary approaches to profile the expression of *A. thaliana* genes as viral infections progressed. First, *A. thaliana* gene expression was assayed in inoculated leaves, systemic leaves, and flowers over a 10-day time course using custom high-throughput fiber optic bead arrays. These arrays represented 388 genes that were primarily selected for these analyses based on a preliminary microarray experiment. Modulation of gene expression was tightly associated with the accumulation of ORMV or TuMV in a given tissue. The genes with altered expression profiles in leaves appear to be distinct from those in flowers. In a second approach, a GFP-tagged virus was used to dissect fluorescent infection foci into four zones expanding out from the center. RNA from each zone was labeled and hybridized to *A. thaliana* ATH1 GeneChips. Differential patterns of expression were observed for over 500 genes that were dependent on virus treatment. The degree to which the expression of these genes was modulated was largely dependent on the relative accumulation of TuMV in each zone, indicating that most effects on host gene expression were cell autonomous. Interestingly, TuMV induced many ribosomal proteins and several components of the 20S core proteasome suggesting that the balance of protein synthesis and turn over is an important component of the host response. Down-regulated genes related to chloroplast functions or cell wall extensibility were also identified and suggest potential mechanisms for TuMV-induced symptoms in A. *thaliana* plants. Other kinds of plant-pathogen interactions also involve localized interactions between microbes and cells of their hosts, and thus, we expect that the dissection strategy employed here should be effective for studying events associated with the hypersensitive response or compatible interactions between plants and bacterial or fungal pathogens as well as other viruses.

301 Effects of cabbage leaf curl virus infection in Arabidopsis on small RNA pathways

Steven Bernacki, James Moyer, Niki Robertson
North Carolina State University

Cabbage leaf curl virus (CaLCuV) is a DNA geminivirus that causes severe symptoms in Arabidopsis. Removal of the coat protein gene attenuates viral symptoms and allows the insertion of up to 800 nt of foreign DNA that can be used to silence specific host genes by virus-induced gene silencing (VIGS). We previously demonstrated that RDR6 and SGS3 are required for VIGS from CaLCuV (Muangsan et al., Plant J. 2004). Here, we analyze the role of Hen1 by using CaLCuV to simultaneously silence Hen1 and ChlI, a visual marker for endogenous gene silencing. ChlI silencing was reduced when Hen1 was simultaneously silenced, a result also found in rdr6 and sgs3 mutants. Unlike rdr6 and sgs3, there was no increase in viral symptoms. The attenuation of ChlI silencing when ChlI and Hen1 were both inserted to the CaLCuV vector could not be attributed to silencing two genes instead of one, because CaLCuV:GFP/ChlI-silenced plants showed visible silencing similar to CaLCuV:ChlI-silenced controls. A relationship between viral symptoms and miRNA pathways has been suggested because miRNAs are often upregulated in virus-infected plants. Similar to other RNA viruses, CaLCuV-infected Arabidopsis plants show increased levels of Dcl1 and the miRNA that regulates it, miR162. This increase was maintained even when CaLCuV was used as a silencing vector for ChlI. Surprisingly, when both Hen1 and ChlI were silenced by CaLCuV, miR162 levels returned to normal. This could be due to the lack of stability of miR162 in the absence of methylation by Hen1. Because symptoms were not attenuated in CaLCuV:ChlI/Hen1-silenced plants compared to CaLCuV:ChlI-silenced plants, our results raise the possibility that increased accumulation of at least some miRNAs may not be directly related to viral symptom formation. This project was supported by Initiative for Future Agriculture and Food Systems Grant no. 2001-52101-11507.

302 Characterization of Chromatin Surrounding Regions of Gene Expression within the Arabidopsis Centromere

Andrew Cal, Song Luo, Daphne Preuss
University of Chicago

Despite a generally repressive heterochromatic environment, expressed genes have been localized to all 5 genetically defined centromeres (Copenhaver et al 1999), and within the Cenp-A binding region of both rice centromere 8 (Nagaki et al 2004) and a human neocentromere (Saffery et al 2003). We hope to gain understanding of how transcription proceeds in the centromere by studying the chromatin environment surrounding centromeric regions of gene expression. DNA methylation profiling by southern blot showed local hypomethylation within the regions. The expressed genes are also associated with markers of open chromatin(acetylated histone H3, dimethylated histone H3 lysine 4) by ChIP assays, and the heterochromatic mark dimethylH3 K9 is excluded from the hypomethylated regions, though it is sometimes associated with flanking sequences. DNA methylation at the nucleotide level by bisulfite sequencing revealed distinct methylation transitions 200-600 bp upstream from the transcriptional start site of all 5 genes examine. These sequences are currently being tested for their ability to block heterochromatin spread.

303 Regulation of *AGAMOUS* Expression by Histone Methylation

Joyce Cartagena[1], Daisuke Kurihara[1], Satoru Fujimoto[1], Yoshitaka Azumi[2], Susumu Uchiyama[1], Sachihiro Matsunaga[1], Kiichi Fukui[1]

[1]Osaka University, [2]Kanagawa University

Plant SET domain proteins are known to be involved in the epigenetic control of gene expression during plant development. In *Arabidopsis*, SET domain proteins are assigned to four classes, namely E(Z), SU(VAR)3-9, TRX and ASH1, based on the homology of their SET domains. Here we report the potential role of an ASH1-related protein, SML (stamen loss), in regulating the expression of the floral homeotic gene, AGAMOUS, and its function in stamen development and differentiation. A loss-of-function phenotype of sml plants is characterized by defects in stamen initiation and development. Furthermore, the level of *AG* expression was significantly reduced among sml flowers. Using chromatin immunoprecipitation assays, it was revealed that SML does not directly bind to the regulatory regions of the *AG* gene. Instead, it was shown that SML regulates *AG* expression by methylation of specific lysine residues. Based on the functional and expression analyses performed on SML, we showed that SML is capable of regulating floral homeotic genes in *Arabidopsis*, particularly those involved in specification of whorl 3 floral organs.

304 Two-step recruitment of RNA-directed DNA methylation to tandem repeat sequences

Simon Chan[1, 3], Yana Bernatavichute[1], Xiaoyu Zhang[1], Carey Li[1], Steven Jacobsen[1, 2]

[1]Dept of MCD Biology, UCLA, [2]Howard Hughes Medical Institute, [3]current address: Section of Plant Biology, UC Davis

DNA methylation silences transposons, but also regulates expression of a select group of endogenous genes. Arabidopsis FWA is silenced in adult tissues by DNA methylation on two tandem repeats, and a new copy of FWA transformed into plants is an efficient target for de novo DNA methylation. We have used FWA transformation as a model to study the initiation of DNA methylation, and have found that de novo DNA methylation is guided by siRNAs produced by an RNA interference (RNAi) pathway. Little is known about why FWA is recognized by the cell as a target for gene silencing. We show that tandem repeats are necessary and sufficient for de novo DNA methylation of FWA, and that repeated character rather than intrinsic sequence is important. Endogenous FWA can adopt either of two stable epigenetic states, methylated and silenced or unmethylated and expressed. Surprisingly, we found siRNAs associated with FWA in both states. The unmethylated endogenous fwa gene was able to recruit siRNA producing machinery, but could not easily regain DNA methylation. Preexisting CG DNA methylation was required to recruit further RNA directed DNA methylation to endogenous FWA, or to cause efficient de novo methylation of transgenic FWA. This suggests that tandem repeats are sufficient to recruit siRNA production, but a second event is required to recruit self-reinforcing DNA methylation factors acting in cis or in trans. Tandem repeats throughout the Arabidopsis genome produce siRNAs, suggesting that repeat acquisition may be a general mechanism for the evolution of gene silencing.

305 Role of HD2 family in Seed Development/Embryogenesis

Adam Colville[2, 1], Tim Xing[2], Brian Miki[1]

[1]Agriculture and Agri-Food Canada, Ottawa, ON, CAN , [2]Carleton University, Ottawa, ON, CAN

Histone modification plays a critical role in maintaining and altering the epigenetic state of the cell. This process is carried out by the acetylation/deacetylation of core histones of a nucleosome enabling the temporal and spatial expression of distinct genes required for normal plant growth and development. The plant kingdom contains the HD2 family of histone deacetylases, bearing no sequence similarity to previously characterized histone-modifying enzymes. Arabidopsis thaliana genome contains 4 HD2 family members (HD2A, HD2B, HD2C, HD2D). In situ hybridization of HD2A, B and C show high levels of expression in ovules, embyros, shoot apical meristem and primary leaves. HD2A suppression (missense) and overexpression lines showed pleiotropic developmental abnormalities including aborted seed development indicating a possible role of HD2A in seed and embryo development.

Analysis of HD2 suppression (RNAi) lines for all HD2 genes showed no visible phenotype. Single loss-of-function mutant lines for HD2A and HD2C, the two most structurally similar HD2s each with three domains were identified but showed no visible phenotype indicating possible functional redundancy. Double mutant of hd2a and hd2c loss-of-function mutants are identical to wildtype until embryogenesis and seed development. Siliques contain ~23.5% seeds with an abnormal appearance and 27% of progeny failed to germinate. Analysis of these seeds found embryos arrested at all major stages of embryogenesis (globular, heart, torpedo and bent-cotyledon).

306 The Nuclear Actin-Related Protein ARP6 is a Pleiotropic Developmental Regulator Required for the Maintenance of FLOWERING LOCUS C (FLC) Expression and Repression of Flowering in Arabidopsis

Roger Deal, Muthugapatti Kandasamy, Elizabeth McKinney, Richard Meagher

Department of Genetics, University of Georgia, Athens, GA

Actin-related proteins (ARPs) are found in the nuclei of all eukaryotic cells, but their functions are generally understood only on the basis of their enigmatic presence in various yeast and animal chromatin-modifying complexes. Arabidopsis thaliana ARP6 is a clear homolog of S. cerevisiae ARP6, which was identified as a component of the SWR1 chromatin remodeling complex. The yeast SWR1 complex deposits the conserved histone variant H2A.Z into euchromatic regions, where it acts to antagonize the encroachment of silent heterochromatin or to poise quiescent promoters for full activation. In order to address the function of ARP6 in Arabidopsis we have examined the subcellular localization, expression patterns, and loss-of-function phenotypes for this protein. We found that Arabidopsis ARP6 was localized to the nucleus and a large part of the cellular pool of this protein was engaged in a high molecular weight complex. ARP6 expression was observed in all vegetative tissues and in a subset of reproductive tissues. Null mutations in ARP6 resulted in a multitude of defects including altered development of the leaf, inflorescence, and flower, as well as reduced female fertility and early flowering in both long- and short-day photoperiods. The early flowering of arp6 mutants was associated with reduced expression of the central floral repressor gene FLOWERING LOCUS C (FLC), as well as MADS AFFECTING FLOWERING 4 (MAF4) and MAF5. In addition, arp6 mutations suppressed the FLC-mediated late flowering of a FRIGIDA (FRI) expressing line, indicating that ARP6 is required for the activation of FLC expression to levels that inhibit flowering. Together these results indicate that ARP6 acts in the nucleus to regulate multiple aspects of plant development, and that it does so at least in part by regulating the expression of developmentally important genes. We discuss evidence for the existence of a SWR1-like complex in plants and present a model for how the H2A.Z variants might serve to control epigenetic switches during plant development.

307 Genetic Control of the Interploidy Hybridization Barrier

Brian Dilkes, Isabelle Henry, Renee Weizbauer, Luca Comai
Biology Department, University of Washington, Seattle WA 98195

Parental contributions to seed development are dramatically asymmetrical. This asymmetry is manifest in the failure of some crosses involving gametes of different ploidy. Multiple mechanisms have been proposed to explain this phenomenon including nuclear to cytoplasm communication, parent-dependent epigenetic imprinting, and gametophytic effects. We are analyzing the genetic control of the interploidy barrier in Arabidopsis using natural variation and gene knockouts. We identified variability for survival of seeds following paternal excess (diploid x tetraploid) crosses in Arabidopsis thaliana. Variation in maternal tolerance was mapped in two RIL populations (Ler x Col and Ler x Cvi). In the Ler x Col RILs, a main effect QTL, Dr. Strangelove, controlled 15% of the phenotypic variation. Genetic analyses of this QTL in segregating populations has confirmed this large effect and fine mapping refined our estimate of QTL position. Mutants of candidate genes have been tested. Diploid loss of function mutations in the WRKY transcription factor ttg2, increased seed viability in paternal excess crosses by three-fold. Variability in the paternally-encoded determinant of interploidy seed failure has also been tested in both RIL and backcross populations of 4x Col x Wa-1. A single locus of major effect was identified on chromosome 1. We are currently testing mutations in candidate genes within the QTL interval for their effect on the pollen determinant of this ploidy barrier.

B.P.D. and L.C. alternate address: UC Davis Genome Center, University of California at Davis, Davis CA 95616

308 Analysis of the functional interaction between CO and TFL2 at the *FT* locus

Sara Farrona, George Coupland, Franziska Turck
Max Planck Institute for Plant Breeding Research

FT plays a main role in the regulation of the transition to reproductive development in Arabidopsis thaliana. The gene is able to integrate the inputs from different flowering pathways, such as the photoperiod, vernalization and autonomous pathway. One component of the photoperiod pathway is the CO gene, which encodes a protein containing zinc-finger and CCT domains. The accumulation of the CO protein in long day conditions promotes the up-regulation of FT expression. It has been proposed that CO acts like a transcription factor with FT as direct target. Since attempts to demonstrate a physical interaction between CO and the FT locus have so far been unsuccessful, the exact mechanism of FT regulation by CO remains unknown.

Another regulator of FT expression is TFL2/LHP1/TU8. This gene encodes a protein with two characteristic domains: a cromo- and a chromoshadow domain. It has been characterized as the only Arabidopsis homologue of mammalian and Drosophila HP1. HP1 protein has been described as component of the heterochromatin although recently a wider role in chromatin structure has been proposed. In Arabidopsis, TFL2 is expressed in the SAM and RAM, in the hypocotyl, and, as FT and CO, in the vascular tissue of young leaves and cotyledons. In a wild type plant CO is required for transcriptional activation of FT, whereas FT is constitutively expressed in the vasculature of tfl2/lhp1 mutant plants. The data indicate that CO counteracts the function of TFL2 at the FT locus.

In order to study the mechanistic interplay between TFL2 and CO, we obtained different transgenic lines: epitope tagged TFL2 (35S::TFL2-HA), CO overexpressor line (35S::CO) and a double transgenic that express both constructs. We carried out chromatin immunoprecipitacion experiments in each of these lines with antibodies against the HA epitope to analyse the binding of TFL2-HA protein in a long region that contains the FT locus and the neighbouring genes. These and further results will be presented at the meeting.

309 Identification Of A Pyridoxine 5′-Phosphate (PNP)/Pyridoxamine 5′-Phosphate (PMP) Oxidase (PDX3) Involved In The Vitamin B6 Salvage Pathway In Arabidopsis thaliana

Eugenia Gonzalez, Margaret Daub
North Carolina State University

PNP/PMP oxidase is a key enzyme in the formation of pyridoxal 5'-phosphate (PLP), the active coenzyme of vitamin B6, essential in many aspects of amino acid metabolism. PLP can be synthesized de novo or by a salvage pathway. In Escherichia coli, PNP, the direct biosynthetic intermediate of PLP, is synthesized de novo by a pathway containing two genes, PdxA and PdxJ, and then oxidized by the PNP/PMP oxidase encoded by PdxH to form PLP. In plants, PLP is the initial product of a different de novo pathway that involves two genes PDX1 and PDX2. In the salvage pathway in E. coli, two kinases have been identified. PN/PM/PL kinase (PdxK) phophorylates pyridoxine (PN), pyridoxime (PM), and pyridoxal (PL) to form PNP, PMP, and PLP, respectively. PL-specific kinase (PdxY) specifically phosphorylates PL. To date in Arabidopsis, the only vitamin B6 salvage pathway enzyme that has been identified is a PL kinase, encoded by the salt overly sensitive 4 (SOS4) gene. In this study we identified a PNP/PMP oxidase (PDX3) involved in the vitamin B6 salvage pathway in Arabidopsis by sequence homology. To confirm the function of this enzyme, the Arabidopsis oxidase gene (At5g49970, renamed PDX3) was cloned and transformed into E. coli pdxH mutants that are unable to grow on minimal medium unless amended with PL. PDX3 complemented the pdxH E. coli mutants, indicated by the ability of the transformed mutants to grow on minimal medium without PL. Two different homozygous T-DNA insertion mutants of A. thaliana ecotype Columbia were recovered for PDX3. Total vitamin B6 and the levels of PN, PM, PL, PMP, and PLP produced by pdx3 mutants, a sos4 mutant and the wild type were determined by a yeast bioassay and HPLC. Surprisingly, sos4 mutants showed a 6.4-fold increase in the amount of PLP, reflected in a 2.6-fold increase in total vitamin B6 compared to the wild type. The pdx3 mutants and the wild type plants showed similar levels of PN, PM, PL, PMP, and PLP and total vitamin B6. Regulation of PDX1, SOS4 and PDX3 in the pdx3 and sos4 mutants and wild type was also determined by quantitative real time PCR. SOS4 was significantly up-regulated in the pdx3 mutants and PDX3 remained unaffected in the sos4 mutants. PDX1.2 was highly express in all mutants. In summary, we have confirmed the identity of a gene encoding an enzyme with PNP/PMP oxidase activity involved in the vitamin B6 salvage pathway of Arabidopsis. The HPLC and gene regulation results suggest that the vitamin B6 salvage pathway in Arabidopsis operates differently than in E. coli.

310 Trapping Enlightening crossovers(X) with Anchored Selectable markers (TEXAS Mapping)

Greg Hatlestad, Alan Lloyd
University of Texas at Austin

Map-based cloning of chemically induced mutations in Arabidopsis thaliana can be a long and tedious process. Because crossover events occur randomly and are invisible until the DNA is analyzed this system requires vast amounts of time and resources. One is forced to screen hundreds to thousands of plants for DNA polymorphisms to find crossover events in useful positions near the mutation. To increase efficiency of fine mapping a mutation we have designed a system to phenotypically identify plants with useful crossover events. The mutant line to be mapped is crossed to T-DNA lines with known inserts in the general area of the unknown mutation. The F2 generation is screened for plants with the T-DNA's dominant selectable marker and the homozygous recessive mutant phenotype. All progeny that pass this screen will have an informative crossover event trapped between the T-DNA and the genetic lesion. A collection of such individuals should have crossovers randomly distributed within the informative interval. This will allow the lesion-containing interval to be narrowed and allow the calculation of the recombinational distance between the T-DNA and the lesion. To test this procedure we are mapping the mutations in 2 known (ttg1-1 and ettin) and 3 unknown genes.

311 Centromeric Retrotransposons: Potential Role of the Integrase C-terminus in Determining Genomic Distribution

Yi Hou, Xiang Gao, Daniel Voytas
Iowa State University

The plant CR retrotransposons are a highly conserved family of Ty3/gypsy elements found almost exclusively in centromeric heterochromatin. This biased genomic distribution may be caused by targeted integration of CR retrotransposons into centromeric regions or by selection against insertions into other chromosomal sites. A variant of the chromodomain (a chromo-like domain or LCHD) was identified in the C-termini of CR retrotransposon integrases. Chromodomains recognize specific modifications on histones, and through this recognition, they direct chromodomain-bearing proteins to specific chromatin domains. We cloned this LCHD and fused it to YFP. In vivo localization experiments showed a specific sub-nuclear distribution of YFP foci that were coincident with the localization of LPH1, a chromodomain-containing protein from Arabidopsis. The LCHD foci also overlapped with the centromere-specific protein CENPC. The sub-nuclear foci became more diffuse in ddm1 mutant leaf cells, which have altered patterns of histone and DNA methylation in heterochromatin. Our data suggest that the C-termini of CR retrotransposon integrases interact with specific components of centromeric heterochromatin. We hypothesize that this interaction underlies the novel distribution pattern of the CR retrotransposon insertions in the genome.

312 A Disrupted MicroRNA Target Site Reveals Novel Regulatory Functions of a Transcription Factor During Plant Development

Clayton Larue[2], Jiangqi Wen[1], John Walker[2]
[1]The Samuel Roberts Noble Foundation, Ardmore, OK, [2]University of Missouri, Columbia, MO

Regulation of development by microRNA (miRNA)-mediated stability of gene transcripts has become a common theme in both plants and animals. miRNAs target ~21 nucleotide sites of mRNAs to direct degradation or disrupt their translation. We identified a semi-dominant mutant, syl for styleless, that shows defects in leaf blade margins, internode patterning and fruit morphology in an EMS mutagenized Arabidopsis population. A single basepair mutation was found by map-based cloning in the miRNA target site of a transcription factor, CUC2. This mutation results in an overaccumulation of transcript in the mutant plant. A mutation in the miRNA corresponding to the mutation in the target site results in a reversion to a wild-type phenotype. This miRNA target site mutation in an endogenous gene may provide interesting insights into the roles miRNAs play in plant development.

C.T. Larue and J. Wen contributed equally to this work.

313 Basis for CACTA Transposon Immobilization by DNA Methylation

Asuka Miura, Miyuki Nakamura, Masaomi Kato, Testuji Kakutani

Department of Integrated Genetics, National Institute of Genetics, Mishima, Japan

Transposon and their derivatives comprise the major components of heterochromatin, and they are often highly methylated. Our previous studies have shown that endogenous transposon CACTA1 is mobilized in the DNA hypomethylation mutant ddm1 (Decrease in DNA Methylation) or in the mutant of DNA methylatransferase genes, suggesting that DNA methylation is necessary for the immobilization of this transposon in wild type (Miura et at. 2001, Nature 411, 212-214; Kato et al. 2003, Curr. Biol. 13, 421-426). The immobilization effects by DNA methylation can be trans-acting (such as transcriptional repression of transposase gene) or cis-limited (such as inhibition of transposase access by heterochromatin formation).

In order to distinguish the possible cis-limited and trans-acting components, we used autonomously mobile CACTA1 and non-autonomous CACTA2. CACTA2 has internal deletion and did not transpose in the absence of the autonomous CACTA1. CACTA1 transposes by itself when activated by the ddm1 mutation. CACTA2 transposed only when both CACTA1 and CACTA2 were derived from ddm1. Notably, CACTA2 derived from wild type did not transpose even in the presence the ddm1-derived CACTA1. Moreover, we examined the transposition of CACTA element in transgenic lines with 35S-CACTA-GUS construct. Mobility of the non-autonomous CACTA element inserted between 35S and GUS reporter was monitored in the presence of the ddm1-activated CACTA1. When the mobility was examined for various 35S-CACTA-GUS lines in the background of ddm1(-/-) and ddm1(-/+), they displayed variable GUS staining patterns depending on their insert locus. These results suggest that the immobilization by DNA methylation is mediated not only through trans-acting but also through cis-limited effects. In order to identify the target sequences of the cis-limited immobilizing effects of DNA methylation, we are currently investigating the immobilizing effect of RNA-induced DNA methylation (RdDM) in various parts of CACTA1 and flanking regions.

314 Silencing Of A Basic Chitinase Gene In Arabidopsis Thaliana Using Short Hairpin-Polymerase III Vector Based System

Ewelina Rodakowska, Marta Derba, Przemyslaw Wojtaszek

Department of Molecular and Cellular Biology, Adam Mickiewicz University, Miedzychodzka 5, 60-371 Poznan, Poland

Chitinases (EC 3.2.1.14) are ubiquitous enzymes hydrolyzing ß-1,4-glycosidic linkages between adjacent N-acetyl-D-glucosamines. There are 25 genes coding for chitinases and chitinase-like proteins in Arabidopsis and they are grouped in the families 18and 19 of glycosyl hydrolases. Plant chitinases are usually considered as pathogenesis-related proteins, although their involvement in stress responses is also well known. However, growing evidence indicates that at least some representatives participatein the regulation of plant growth and development; they are involved in nodulation, PCD and somatic embryogenesis; in the last case probably through interactions with wall-associated arabinogalactan proteins. We are interested firstly in the function of chitinases in plant morphology and development and secondly in short hairpin (shRNA) methodology - a new approach in plants reverse genetics. For theanalysis, a basic chitinase gene (At3g12500) has been chosen. It is thought to be a pathogenesis-related protein, since its expression level increases upon pathogen attack. On the other hand, its expression in rootsis constitutive during whole plants life. Transgenic plants with decreased expression of chitinase mRNA have been obtained using short hairpin approach. In this communication, the generation of transgenic plants with silenced chitinase gene, and preliminary phenotype analysis will be presented. This work is funded by the Ministry of Education and Science grant PBZ-KBN-089/P06/2003 to P.W.

315 Finding Intron Sequences That Enhance Gene Expression

Alan Rose, Ian Korf

Molecular and Cellular Biology, University of California, 1 Shields Ave., Davis, CA 95616

Many introns increase gene expression in diverse organisms by mechanisms that are poorly understood but are unlike enhancer elements. Because efficiently spliced introns differ widely in their ability to stimulate expression, splicing alone is not sufficient for intron-mediated enhancement (IME), and there must be sequences that differ between introns that determine the magnitude of enhancement. To locate these sequences, a series of deletion-containing and hybrid introns were tested in a fusion between the TRP1 gene and GUS in transgenic Arabidopsis. All of the deletion-containing introns stimulated mRNA accumulation as much as did the full-length intron. Similarly, hybrids in which either the 5' or 3' half of a strongly enhancing intron were fused to the other half of a 'weak' intron appear to be as effective as the entire 'strong' intron. Thus, enhancing sequences are redundant, dispersed, and more prevalent in some introns than others. To identify sequences that fit these criteria, an algorithm was devised that determines how well a test intron matches the pentamer sequence profile of all the introns in genes whose expression is predicted to be in the top 20% of Arabidopsis genes, based on codon entropy. Three findings suggest that this algorithm (the IMEter) may be relevant to IME. 1) The score generated by the IMEter is proportional to the degree to which each of six introns elevate TRP1:GUS expression. 2) Of the 15 Arabidopsis introns shown by other labs to enhance expression, virtually all generate an IMEter score of 10 or more, even though less than 2.8% of all Arabidopsis introns yield a score this high. 3) Average IMEter scores of introns separated by their ordinal number drop from first introns to sixth introns and then level off. This pattern is in striking agreement with the finding that the ability of an intron to stimulate mRNA accumulation declines with distance from the promoter until it is lost entirely around 1 Kb from the start. We are testing the importance of the sequence CGAT, identified using the IMEter, by asking if altering the abundance of this motif changes the enhancing ability of introns.

316 Genetic and Epigenetic Study of de novo Centromere Formation in Arabidopsis thaliana

Jay Shrestha, Song Luo, Daphne Preuss

University of Chicago

Centromeres play a critical role in ensuring proper segregation of chromosomes by specifying site for kinetochore assembly and spindle attachment during cell division. Our lab is interested in studying the genetic and epigenetic determinants at the centromere that specify its function in Arabidopsis thaliana. The primary question this study aims to answer is: what genetic elements in the centromeric region of Arabidopsis thaliana are sufficient to confer centromeric activity. To this end, DNA fragments from different regions of the Arabidopsis thaliana genome are being integrated into the chromosome by Agrobacterium-mediated transformation. These DNA fragments include BAC clones carrying centromeric DNA repeats, gene rich euchromatic sequences, heterochromatic DNA from the NOR, and the chromosome 4 knob. Following integration of these DNA fragments into the chromosome, de novo formation of centromeres at these inserted sequences will be investigated by using various genetic, cell and molecular biological methods. The candidates that show de novo centromere formation will be further analyzed for epigenetic changes at the new centromeric site. Studying these candidate centromeric fragments can give us an insight into the size and sequence requirement for centromere function in Arabidopsis thaliana.

317 Using Synthetic RPP8 Gene Clusters To Model R Gene Evolution By Meiotic Unequal Crossing-Over

Stacey Simon, Bonnie Woffenden, Crystal Gilbert, John Jelesko, John McDowell
Virginia Tech

Disease resistance genes (R genes) are frequently organized as gene clusters. Unequal crossing-over between different linked genes of a cluster can create new combinations of R genes, as well as chimeric genes, thereby facilitating the evolution of new R genes. The resulting chimeric R genes could have an altered pathogen recognition specificity. The Arabidopsis RPP8 gene, for downy mildew resistance, belongs to a two-gene cluster, and sequence comparisons suggest that unequal crossing-over has significantly affected the evolution of allelic diversity at RPP8. An allelic series of three different pathogen recognition specificities has been defined at the RPP8 locus. We are utilizing a genetic screen to model both the frequency and character of unequal crossing-over within a synthetic RPP8 transgenic cluster. We will identify rare meiotic unequal cross-over events by coupling chimeric gene formation to the activation of the Firefly Luciferase gene. The recombination breakpoints will be mapped and the pathogen resistance specificities of the chimeric RPP8 genes will be tested. We will also address whether the frequency of meiotic recombination is affected by abiotic and biotic stress. This study will provide general insights into the frequency and character of meiotic unequal crossing-over and its impact on the evolution of functional diversity within R gene clusters.

318 PAG1, the α7 Subunit of the 20S Proteasome, is Essential in Pollen Development

Gulsum Soyler-Ogretim, Jed Doelling
Division of Plant and Soil Sciences, West Virginia University, Morgantown, 26506

The 26S proteasome is responsible for the degradation of ubiquitin-tagged proteins in eukaryotic organisms. 20S core particle of the 26S proteasome consists of 4 stacked rings of 7 proteins each: the two inner rings are each composed of 7 different β subunits and two outer rings are each composed of 7α subunits. Protein degradation by the proteasome is tightly regulated and occurs inside the cylinder. Whereas two different genes encode many of the α and β subunits in Arabidopsis, there is only one gene that encodes the α subunit PAG1. In this study, we use reverse genetics to study the role of PAG1 and the 26S proteasome during Arabidopsis growth and development.

We acquired a potential PAG1 T-DNA insertion mutant from the Arabidopsis Biological Resource Center based on search of the database www.arabidopsis.org. The presence of T-DNA insertion within PAG1 was confirmed by PCR genotyping. Because homozygous mutant plants were not found among the progeny of heterozygous plants, reciprocal crosses between a heterozygous mutant and a wild type plant were conducted to determine the cause. When the heterozygous plant was used as the pollen donor, no heterozygous individuals were identified among 100 random offspring. This suggests that pollen transmission of the mutant allele is hindered: if mutant pollen and wild type pollen are equal, one would expect half of the offspring to contain a mutant allele. Ovule transmission of the mutant allele was found to occur at the expected proportion.

We are continuing to characterize PAG1 mutant individuals by conducting complementation tests using endogenous and inducible promoters and to characterize the development and the function of mutant pollen. The hope is to determine the consequences of defective ubiquitin-dependent protein degradation at different stages of plant development. Pollen development will be monitored using microscopy to count nuclei following DAPI staining and analyzing pollen morphology. Pollen function will be investigated using in vitro pollen germination assays and vital stains.

319 Biochemical Characterization Of Arabidopsis thaliana PPR proteins

Magalie UYTTEWAAL[1], Nadege Arnal[1], Jean Bigeard[1], Anna Debicka[1], Martine Quadrado[1], Jean-Pierre Renou[2], Hakim Mireau[1]

[1]INRA - Station de Genetique - Route de Saint-Cyr F78026 Versailles cedex France, [2]INRA - URGV - 2, rue Gaston Cremieux 91057 Evry cedex

The expression of plastid and mitochondrial genomes is dependent on a large number of nucleus-encoded factors that were shown to act predominantly at a posttranscriptional level. Genetic studies, carried in maize and Arabidopsis and positional cloning of several cytoplasmic male sterility restorer genes (in rice, petunia and radish) revealed a predominant involvement of PPR proteins in plant organellar RNA expression. They have been genetically linked to various processes like RNA stabilization, processing, translation and editing. PPR proteins have been identified in various eukaryotes but this protein family has literally exploded in higher plants, with over 450 members in Arabidopsis and rice. It was hypothesized that such expansion could be correlated with the apparition a specific function in plant posttranscriptional processes like RNA editing. This large gene family is characterised by the presence of tandem arrays of a 35-aminoacid motif. Because of its structural similarity to the TPR (tetratricopeptide repeat) motif known to form interacting domains, the PPR motif is proposed to constitute highly specific RNA binding domains that could recruit catalytic protein on a specific RNA sites. It makes no doubt that PPRs are key factors of organellar gene expression but their molecular functions remain to be elucidated. To unravel the molecular roles of some PPR proteins we decided to identify their interacting RNA and protein partners that may display known functions.

As a first step to select appropriate proteins, a biochemical screen looking for PPR proteins engaged in high molecular protein complexes has been realised. About ten Arabidopsis PPR proteins were fused to short epitope tags (3HA, FLAG) and to various versions of TAP tags allowing protein complex isolation. Analysis of stromal and mitochondrial extracts by size exclusion chromatography allowed us to identify 5 PPR proteins involved in multi-protein complexes. Three PPRs have been purified by tandem affinity purification and their putative partners needs to be identified by mass spectrometry. To identify putative RNA associated to these proteins, we envisage to use a microarray-based strategy recently developed on the maize CRP1 protein (method called RIP-Chip standing for RNA immunoprecipitation and chip hybridization). In our case, the immunoprecipitation assays will be carried on the tagged selected PPRs and the coimmunoprecipitated RNA will be hybridized on microarray slides covering the Arabidopsis mitochondrial and chloroplast genomes. Obtained results will be presented.

320 Genome-wide High Resolution Mapping and Functional Analysis of DNA Methylation in *Arabidopsis thaliana*

Xiaoyu Zhang[1], Junshi Yazaki[2], Ambika Sundaresan[2], Simon Chan[1], Huaming Chen[2], Lianna Johnson[1], Paul Shinn[2], Hiroshi Shiba[2], Shawn Cokus[1], Matteo Pellegrini[1], Steve Jacobsen[1, 3], Joseph Ecker[2]

[1]Dept. MCDB, UCLA, [2]The Salk Institute for Biological Studies, [3]The Howard Hughes Medical Institute, UCLA

Cytosine DNA methylation is a conserved epigenetic silencing mechanism involved in many important biological processes, including defense against transposons and other invading DNA, maintenance of chromosomal structure and genome stability, establishment of parental imprinting, and regulation of gene expression. Previous studies have largely focused on the establishment and maintenance of DNA methylation as well as its role in controlling individual genes. However, a genome-wide analysis of DNA methylation or its function in regulating gene expression has not been performed for any organism, thus greatly limiting our understanding of this important mechanism. Here we describe the first such analysis using the model plant Arabidopsis. Methylated and unmethylated DNA were separated by two biochemical methods and hybridized to whole-genome tiling microarrays, which allowed the genome-wide identification of methylated regions with high resolution. Methylated DNA comprises ~20% of the Arabidopsis genome and is highly enriched in heterochromatin. Methylation was also found in over 1/3 of all Arabidopsis genes and its distribution is severely biased towards the 3' end, whereas promoters are hypomethylated. Furthermore, methylated and unmethylated genes have significantly different expression levels and tissue-specificity. Expression profiles were determined using the same microarray platform for mutants severely impaired in DNA methylation. Drastic activation of transposons was observed, as well as large-scale changes in gene expression, antisense transcription and intergenic non-coding RNA accumulation.

321 On the diversity of the Arabidopsis Fructokinase Gene Family

Borjana Arsova, Wolfgang Lein

Max-Planck-Institute of Molecular Plant Physiology, Wissenschaftspark Golm, Am Muhlenberg 1, 14476 Potsdam, Germany

In the post genomic era it is obvious that we know little or nothing about the majority of genes that are contained in the recently sequenced genomes. Thus, the challenge exists to attach functions to the numerous genes of poorly described or totally unknown function.

Also, the existence of multiple enzyme isoforms, whose precise properties and subcellular localizations are still unresolved, disables the proper understanding of the structure and regulation even of basic metabolic pathways, such as glycolysis, with the possibility that multiple isoforms provide the regulatory framework that is needed to adapt metabolism during development and in response to the environment.

Free fructose, resulting e.g. from sucrose cleavage, is phosphorylated by Fructokinase (FK) providing fructose-6-phosphate which can then be used for starch synthesis or glycolysis. The inhibition of this enzyme by free Fructose plays an important role in maintaining the flux of carbon towards starch formation, thus having a regulatory role.

Surprisingly, the Arabidopsis-genome encodes 10 putative FK-isoforms (AtFK), which phylogenetically can be divided into three subgroups. All related literature for FK-isoforms from maize, rice and tomato where biochemical analysis is linked to individual genes belongs to two, closely related subgroups. In contrast, the third group represented by a distantly associated cluster, containing two AtFK isoforms, remains uncharacterised in this or any other species. Sequence analysis showed, that all but one AtFK gene are carrying all described conserved protein motives (1), that are characteristic for a fructokinase. Although the in silico analysis of expression patterns in tissues and organs (2) didn't show a specific spatial separation, it did point to one isoform as the predominant form. Similarly we could identify diurnally and substantially expressed forms, while a co-response analysis (3) allowed us to predict three of these forms as plastid localized (including the two AtFKs from the third cluster, one of which was proved to be plastid localised by an independent group(4)). This in itself was an interesting finding because a plastidial fructokinase has not been described so far. Only two of the AtFKs showed co-expressing glycolysis related genes.

Recent results that will be presented include: biochemical analysis of 5 recombinant proteins (including the putative palstidial forms) - all showing FK but not HK activity, assays querying the intracellular localisation of the proteins etc.

(1) Pego & Smeekens, 2000, Tips; (2) Steinhauser et al, 2004, Bioinformatics; (3) Thimm et al, 2004, Plant J; (4) Kleffman et al. (2004), Curr. Biol

322 Transcriptomic and Metabolomic Analysis of Antisense ATP-Citrate Lyase *Arabidopsis thaliana* Supplemented with Malonic Acid

Heather Babka, Beth Fatland, Basil Nikolau, Eve Syrkin Wurtele

Iowa State University

ATP-citrate lyase (ACL) catalyzes the production of acetyl-CoA in the cytosol of *Arabidopsis thaliana*. The cytosolic pool of acetyl-CoA is required for the production of the stress-related phytochemicals, stilbenoids and flavonoids, and for elongation of fatty acids. In addition, cytosolic acetyl-CoA is essential for the synthesis of membrane sterols. Antisense ACL plants have reduced ACL activity, and a very distinct phenotype including miniature organs, smaller cells, reduced cuticular wax, and an increased accumulation of starch. This phenotype is reversed by exogenous malonic acid, which feeds into the carboxylation pathway of acetyl-CoA metabolism (Fatland et al., 2004). Antisense ACL and wildtype plants with and without treatment with malonic acid were analyzed by transcriptomics and metabolomics. These analyses are providing clues as to the mechanisms that Arabidopsis employs to cope with a decreased level of ACL activity *in planta*.

323 Fluorescent amino acid sensors report amino acid dynamics in living cells

Martin Bogner, Uwe Ludewig

Zentrum fuer Molekularbiologie der Pflanzen (ZMBP), Pflanzenphysiologie, Universitaet Tuebingen

All organisms require sufficient cytosolic amino acid levels to maintain protein synthesis at adequate rate. In addition to their function as building blocks of proteins, amino acids also serve other functions in plants, e.g as nitrogen storage compounds or osmotic protectants. The cytosolic amino acid concentrations differ between organs and are subject to metabolic changes and compartmentation. In order to measure amino acid changes with subcellular specificity in plants, fluorescent proteins have been designed and constructed that respond specifically to changes in the amino acid concentrations. These sensors are based on the bacterial protein QBP from *E.coli*, which is known to commit large conformational changes upon the high-affinity binding of its substrate, glutamine. We attached two different green fluorescent protein variants to QBP, in a way each is conceted to a different hemisphere. Fluorescence resonance energy transfer (FRET) between the attached chromophores was observed. In the purified recombinant protein a change in fluorescence was observed after addition of argenine. Affinity for glutamine could be partially restored by mutations in the binding pocket. This goes well with the computer model which indicates changes in the morphology of the binding pocket, because of the insertion of one GFP varinat into the linear sequence of QBP. Measurements with all proteinogenic amino acids indicated selective FRET changes for different constructs. The sensors were expressed in *E.coli*, in yeast and in plants. Fluorescence changes were observed upon addition of amino acids, in accordance with amino acid changes in the cytoplasm. Fluorescent amino acid sensors appear to be a versatile tool to study the *in vivo* dynamics of metabolism and compartmentation, especially in large-scale genomic approaches and upon environmental changes.

324 Identification and Characterization of a Novel Plastid Envelope Protein

Andrea Braeutigam[1], Susanne Hoffmann-Benning[2], Andreas Weber[3]

[1]Michigan State University, Genetics Program, [2]Michigan State University, Department of Biochemistry and Molecular Biology, [3]Michigan State University, Department of Plant Biology

C4 plants such as maize use a carbon concentration mechanism which renders their carbon fixation more efficient compared to C3 plants. This carbon concentration mechanism requires spatial separation and compartimentation of several enzymatic reactions and thus requires immense metabolite fluxes across the plastid envelope. We hypothesized that we can identify candidates for the transport proteins that catalyze those fluxes by analyzing the proteome of the plastid envelope of maize plastids. Specifically we hypothesize that we can identify candidate genes for the transport of oxaloacetate, malate and pyruvate.

We analyzed the mesophyll plastid envelope by proteomics and identified more than 30 proteins with high confidence. Within the highly hydrophobic protein fraction a band is visible in SDS gels which is absent from the envelopes of C3 plants and we identified a protein with unknown function from this band. This protein is present throughout the plant kingdom including red algae, green algae and land plants but notably absent from cyanobacteria.

To understand the *in planta* function of this novel protein we identified a null allele in Arabidopsis thaliana. A detailed analysis of the phenotype will be presented.

325 Poplar Carbohydrate Active Enzymes. Gene Identification and Expression Analyses

Jane Geisler-Lee[1], Matt Geisler[1], Pedro Coutinho[2], Bo Segerman[1], Nobuyuki Nishikubo[1], Junko Takahashi[1], Henrik Aspeborg[3], Soraya Djerbi[3], Emma Master[3], Sara Andersson-Gunners[1], Bjorn Sundberg[1], Stanislaw Karpinski[4], Tuula T. Teeri Teeri[3], Leszek Kleczkowski[1], Bernard Henrissat[2], Ewa Mellerowicz[1]

[1]Ume? Plant Science Center, Umea, Sweden, [2]Architecture et Fonction des Macromolecules Biologiques, Marseille, France, [3]Royal Institute of Technology, Stockholm, Sweden, [4]Stockholm University, Stockholm, Sweden

Based on sequence homology, about 1,600 genes which encode carbohydrate active enzymes (CAZymes) in the Populus trichocarpa genome were identified, annotated and assembled into families of glycosyltransferases (GTs), glycoside hydrolases (GHs), carbohydrate esterase (CEs), polysaccharide lyases (PLs), and expansins (Exps). A collection of 100,000 expressed sequence tags from 17 different tissues was used to analyze CAZymes gene expression in poplar (Populus spp.) and to compare to microarray data for poplar and Arabidopsis. Based on Fisher's exact test ($p \leq 5\%$), CAZyme families and expansins were shown differentially expressed. The families with the highest levels of tissue-specific expression generally are involved in cell wall carbohydrate biosynthesis and modification, or in starch biosynthesis and turnover. Sucrose synthases and cellulose synthases were the most abundant transcripts specifically expressed in wood-forming tissues. Woody tissues were the most plentiful source of different sucrose synthase and cellulose synthase transcripts which demonstrates their importance in xylogenesis. Little expression of genes related to starch metabolism during wood formation were found, which was consistent with the metabolic flux of carbon to cell wall biosynthesis. The CAZyme transcriptomes in different poplar tissues showed profound changes; this led to some main differences in CAZyme genes and their regulation between herbaceous and woody plants.

326 Functional Evidence for the Involvement of Arabidopsis IspF Homolog in the Nonmevalonate Pathway of Plastid Isoprenoid Biosynthesis

Ming-Hsiun Hsieh[1], Howard Goodman[2]

[1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan , [2]Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA

There are two independent pathways, the cytosolic mevalonate (MVA) pathway and the plastid nonmevalonate (nonMVA) pathway, to synthesize isopentenyl diphosphate and dimethylallyl diphosphate in plants. Carotenoids and the phytyl side chain of chlorophylls are isoprenoids derived from the plastid nonMVA pathway. All enzymes involved in the nonMVA pathway have been identified in Escherichia coli. The E. coli IspF protein catalyzes a unique cyclization reaction to convert 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate into 2-C-methyl-D-erythritol 2,4-cyclodiphosphate in the nonMVA pathway. We have characterized an Arabidopsis T-DNA insertion mutant, ispF-1, that has a null mutation in the IspF gene. Homozygous ispF-1 mutants are albino lethal and the IspF transcripts are undetectable in these plants. Moreover, the ispF-1 mutant chloroplasts are filled with vesicles instead of thylakoids. Amino acid sequence alignment reveals that the IspF proteins are highly conserved between plants and bacteria. Interestingly, expression of the Arabidopsis IspF protein can rescue the lethal phenotype of an E. coli ispF mutant. These results indicate that the Arabidopsis IspF may share similar enzymatic mechanisms with the E. coli protein.

327 The Role of Tryptophan Metabolism in Auxin Homeostasis

Anna Hull[2], Arifa Ahamed[1], Adedamola Adepoju[1], Jeanine Ledoux[1], Jennifer Normanly[1], John Celenza[3], Mike Pieck[3], Judith Bender[4]

[1]Dept. of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, (MA) , [2]Lincoln University, (PA), [3]Boston University, Boston, (MA), [4]Dept. of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, (MD)

Plants synthesize a great variety of secondary metabolites derived from amino acids. Some of these compounds serve as growth regulators, while most function in defense against pathogens. In Arabidopsis thaliana, the amino acid tryptophan (Trp) is a precursor for the growth regulator, IAA, and for two distinct defense compounds: the anti-microbial compound camalexin and the anti-herbivory class compounds called indole glucosinolates.

Two Arabidopsis cytochrome P450s, CYP79B2 and CYP79B3 convert Trp to indole-3-acetaldoxime (IAOx). The cloning of and subsequent genetic analysis of the CYP79B2 and CYP79B3 genes has pointed to (IAOx) being a key metabolite in the production of IAA, indole glucosinolates and camalexin. CYP79B2-overexpressing plants (CYP79B2-OEX) have elevated levels of free IAA and indole glucosinolates while the cyp79B2 cyp79B3 double mutant has decreased free IAA and make no indole glucosinolates or camalexin (Hull et al., 2000; Mikkelsen et al., 2000; Zhao et al., 2002; Glawischnig et al., 2004).

We have also found evidence in Arabidopsis for cross-talk between Trp and IAA primary metabolic pathways and secondary metabolic pathways. A dominant overexpression allele of the Arabidopsis Myb transcription factor ATR1, atr1D, increases flux of Trp into indole glucosinolates by increasing expression of CYP79B2, CYP79B3 and CYP83B1 while resulting in only a modest increase in IAA. In addition, atr1-2 mutants have reduced CYP79B2, CYP79B3, and CYP83B1 transcription and produce approximately 30% reduced indole glucosinolate accumulation in adult leaves compared to WT (Celenza et al., 2005).

Our current goals are to define further the role of IAOx in IAA synthesis by characterizing CYP79B2-mediated IAA synthesis in tobacco and Arabidopsis and to confirm or negate a role for IAOx in IAA synthesis in non-cruciferous plant families. In addition, we wish to identify genes in the IAOx to IAA pathway as well as identifying alternate routes for IAA synthesis. A combination of mutants screens and targeted metabolic profiling is being used to achieve these goals.

Celenza, J.L., Quiel, J.A., Smolen, G.A., Merrikh, H., Silvestro, A.R., Normanly, J., and Bender, J. (2005). The Arabidopsis ATR1 Myb transcription factor controls indolic glucosinolate homeostasis. Plant Physiol 137, 253-262. Hull, A.K., Vij, R., and Celenza, J.L. (2000). Acad. Sci. USA 97, 2379-2384. Zhao, Y., Hull, A.K., Gupta, N.R., Goss, K.A., Alonso, J., Ecker, J.R., Normanly, J., Chory, J., and Celenza, J.L. (2002). Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. Genes Dev 16, 3100-3112.

328 Interaction between SHMT and Fd-GOGAT in photorespiration

Aziz Jamai[1], Patrice Salome[1], Lars Voll[2], Andreas P. Weber[2], C. Robertson McClung[1]

[1]Dartmouth College, Hanover, (NH) USA, [2]Michigan State University, East Lansing, (MI) USA

In the leaves, Rubisco catalyzes the carboxylation of ribulose-1, 5-bisphosphate (RuBP), but it also catalyzes an oxygenase reaction. Photorespiration is a complex series of reactions to salvage the C2 product of the oxygenation of RuBP. Mitochondrial serine hydroxymethyltransferase (SHMT) is one of the key enzymes of this cycle. A mutant (shm1-1) defective in SHM1 function lacks SHMT activity and is unable to grow at ambient CO2 concentrations but grows normally at elevated CO2 concentrations (Voll et al., 2006, Plant Physiol. 140:59).

Here we address the molecular basis of a second mutant, glu1-201, that also lacks photorespiratory SHMT activity. A test of allelism between shm1-1 and glu1-201 indicates that they are two distinct loci. glu1-201 maps to the top of chromosome V in a region that lacks SHM genes. We have established that the GLU1 gene, which encodes Fd-GOGAT, is the defective gene responsible for the glu1-201 phenotype. There is a single nucleotide change between wild type and mutant glu1-201 changing amino acid 1270 from L to F. Introduction of a wild type GLU1 allele under control of either the 35S or the GLU1 promoter into glu1-201 restores wild type levels of SHMT activity and allows growth at ambient CO2 concentrations . However, introduction of the glu1-201 coding sequence driven by the 35S promoter fails to rescue.

Although glu1-201 has a missense mutation in the Fd-GOGAT coding sequence, the mutant is defective in SHMT activity and exhibits wild-type Fd-GOGAT activity. In contrast, a T-DNA insertion mutant, glu1-202, lacks Fd-GOGAT activity and shows reduced SHMT activity. The F1 progeny of a cross between glu1-201 and glu1-202 exhibit loss of SHMT activity and wild-type Fd-GOGAT activity. These data suggest that Fd-GOGAT plays an essential role in mitochondrial SHMT activity that is independent of Fd-GOGAT activity. To test this, we generated a glu1-203 allele with several missense mutations in the catalytic region and showed that it fails to restore wild type Fd-GOGAT activity in the glu1-202 T-DNA mutant, yet fully rescues SHMT activity in the glu1-201 mutant. We obtained similar results with a glu1-204 transgene missing 2 kb of coding sequence, including the catalytic domain, but retaining the C-terminal domain containing L1270. Our results demonstrate that mitochondrial SHMT activity requires the expression of the Fd-GOGAT, although the mechanism by which Fd-GOGAT supports SHMT activity is unknown.

329 Composition of Esters in the Stem Wax of Arabidopsis *cer* Mutants

Christine Lai, Ljerka Kunst, Xuemin Wu, Stephen Greer, Reinhard Jetter
University of British Columbia, Dept. of Botany

Primary plant surfaces are covered by a cuticle consisting of very long chain 'waxes' embedded in and deposited on a fatty acid polyester matrix of 'cutin'. The cuticle serves as a crucial first line of defense against biotic and abiotic stress from the plant's environment. A number of Arabidopsis mutants have been identified that are deficient in cuticle formation and provide important tools for studying the biosynthesis and export of cuticular wax components. Although the wax of these *cer* mutants has been analyzed in some detail, data on the chain length and isomer composition of the alkyl esters are missing to date.

Wax alkyl esters are composed of straight chain, saturated fatty acids bonded to very long chain alcohols. Are the esterified acids and alcohols the same as those found as free compounds in the wax mixture?

For the current study, *cer* mutants with known altered composition of free wax alcohols were selected and their ester composition was determined by GC-FID and GC-MS. Wax esters constituted 0.2 - 0.6 μg/cm² of the mutant wax mixtures. Palmitate was the predominant ester acid, while C22 to C30 alcohols were found esterified. The chain length patterns of free and esterified alcohols matched for all those mutants with alcohol/ester amounts higher than corresponding wildtypes. In contrast, both patterns differed for other mutants with alcohol quantities below the wildtype level. The biosynthetic relevance of these findings will be discussed.

330 Assessment of tocopherol recycling during light stress in *Arabidopsis thaliana*

Naoko Kobayashi, Dean DellaPenna
Michigan State University

Tocopherols (Vitamin E) are lipid-soluble antioxidants synthesized by all plants and cyanobacteria. Genes encoding the main tocopherol biosynthetic pathway have now been isolated primarily from mutant analyses in Arabidopsis thaliana, and used to successfully engineer the content of tocopherols and biosynthetic intermediates in plants. Tocopherols play an important role as antioxidants which results in the generation of various tocopherol oxidation products. In animal systems α-tocopherol quenches and scavenges lipid peroxy radicals and can be reversibly oxidized in a redox cycle or oxidized further to products such as α-tocopherolquione (TQ), epoxy-α-tocopherolquiones, and α-tocopherolhydroquionone (THQ). Unlike animals, plants can synthesize tocopherols and it is therefore possible that tocopherols might be regenerated from oxidation products by dehydrating the 3`position of the phytyl tail of TQ and THQ and then cyclizing the products to reform a chromanol ring. To test whether such a system is operating in plants, we have fed isolated Arabidopsis chloroplasts labeled tocopherol oxidation products and followed the production of any newly formed compounds. The effects of different light intensity (100μmol and 1,300μmol), cofactors (NADPH, ATP and GSH) and different concentration of detergent (deriphat) will be presented.

331 A Putative Bifunctional Wax Ester Synthase / Acyl-CoA:Diacylglycerol Acyltransferase WSD10 is Involved in Stem Wax Ester Synthesis in *Arabidopsis*

Xuemin Wu, Patricia Lam, Reinhard Jetter, David Bird, Lacey Samuels, Ljerka Kunst
Department of Botany, University of British Columbia, 6270 University Blvd, Vancouver, BC, V6T 1Z4, Canada

Neutral lipids, including wax esters and triacylglycerols, have very important dietetic, technical and pharmaceutical applications. A bifunctional wax ester synthase (WS)/ acyl-CoA:diacylglycerol acyltransferase (DGAT) was identified in *Acinetobacter calcoaceticus* via mutant analysis. This enzyme catalyzes the transfer of an acyl group onto a very-long chain alcohol acceptor. On the basis of homology to the WS/DGAT *Acinetobacter* sequence, a gene family of 11 members (*WSD* family) was found in *Arabidopsis*. To date none of the *WSD* genes has been characterized. It is likely, however, that one or more of these genes encode enzymes responsible for the formation of wax esters found in the cuticle, a protective lipid structure deposited on shoot surfaces of all land plants. The cuticle is composed of cutin polymer matrix and waxes. These waxes are arranged into an intracuticular layer in close association with cutin, and an epicuticular film exterior to this, which often includes epicuticular wax crystals.

In this report we provide evidence that one gene member of the *Arabidopsis WSD* family, the *WSD10*, is required for the production of cuticular wax esters. Wax analyses of two independent T-DNA insertion knock-out mutants of *WSD10* demonstrated that there were no detectable wax esters on the stems of these lines. The cryo-SEM examination of the surface of the *wsd10* mutant stem showed altered shape of epicuticular wax crystals. *WSD10*promoter::GUS activity in transgenic *Arabidopsis* lines revealed that, in addition to the stem, this gene is highly expressed in the pedicel, suggesting that WSD10 may also perform acyltransferase functions at this location.

332 Characterization of K^+-dependent and –independent L-asparaginases from Arabidopsis

Frederic Marsolais, Luanne Bruneau, Ralph Chapman
Agriculture and Agri-Food Canada

L-asparaginases (EC 3.5.1.1) are hypothesized to play an important role in nitrogen supply to sink tissues, especially in legume developing seeds. Two plant L-asparaginase subtypes have been previously identified according to their K^+-dependence for catalytic activity. An L-asparaginase homologous to Lupinus K^+-independent enzymes with activity towards β-aspartyl dipeptides, At5g08100, has been previously characterized as a member of the N-terminal nucleophile amidohydrolase superfamily in Arabidopsis. In this study, a K^+-dependent L-asparaginase from Arabidopsis, At3g16150, is characterized. Recombinant At3g16150 and At5g08100 share a similar subunit structure and conserved auto-proteolytic pentapeptide cleavage site, commencing with the catalytic Thr nucleophile, as determined by ESI-MS. The catalytic activity of At3g16150 was enhanced approximately 10-fold in the presence of K^+. At3g16150 was strictly specific for L-Asn, and had no activity towards β-aspartyl dipeptides. At3g16150 also had an approximately 80-fold higher catalytic efficiency with L-Asn relative to At5g08100. Among β-aspartyl dipeptides tested, At5g08100 had a preference for β-aspartyl-His, with catalytic efficiency comparable to that with L-Asn. Phylogenetic analysis revealed that At3g16150 and At5g08100 belong to two distinct subfamilies. Transcript levels of At3g16150 and At5g08100 were highest in sink tissues, especially in flowers and siliques early in development, as determined by quantitative RT-PCR. The overlapping spatial patterns of expression argue for a partially redundant function of the enzymes. However, the high catalytic efficiency suggests that the K^+-dependent enzyme may metabolize L-Asn more efficiently under conditions of high metabolic demand for N.

333 Regulation of Fructose-1,6-Bisphosphate Aldolase via Glutathionylation in Arabidopsis Chloroplasts

Matsumoto Masayoshi, Ogawa Ken'ich
RIBS Okayama

The role of glutathionylation of fructose-1, 6-bisphosphate aldolase (FBA) in chloroplasts was investigated. The *Arabidopsis* genome includes three genes for chloroplastic FBAs, of which glutathionylated FBA was designated as FBA1. Recombinant FBA1 activity had a strongly pH-dependency, which suited stromal pH that changes from 7 to 8 following illumination: FBA1 activity at pH 8 was 2-fold higher than at pH 7. Glutathione (GSH) strengthened this pH-dependency by 250 %. Other FBAs did not have such features.

Thioredoxin (Trx) activates the Calvin cycle, but dithiothreitol and Trx inhibited the activity of three FBAs. At pH 8, GSH reactivated FBA1 only via glutathinonylation. FBA activity in wild-type chloroplasts was regulated by GSH and pH as was FBA1, while that in a T-DNA inserted mutant of FBA1 was little affected by GSH or pH. Altogether, FBA1 is expressed in vivo and regulated via glutathionylation to activate and facilitate the Calvin cycle.

334 The FRO3 Fe(III) Chelate Reductase Plays A Vital Role In Iron Homeostasis In Arabidopsis

Indrani Mukherjee, Erin Connolly
University of South Carolina, Department of Biological Sciences, Columbia, SC 29208

The Arabidopsis FRO2 gene encodes the iron-deficiency inducible Fe(III) chelate reductase responsible for reduction of iron at the root surface; subsequent transport of iron across the plasma membrane is carried out by a ferrous iron transporter (IRT1). Seven additional FRO genes are present in the Arabidopsis genome and our current studies are aimed at determining the functions of each FRO family member. After iron is taken up by root cells, it is thought that iron is re-oxidized to the ferric form and is transported as Fe(III)-citrate via the xylem to the aerial parts of the plant. Fe(III) chelate reductase activity is required for further iron uptake by leaf cells; FROs may also function in reduction of iron at various organellar membranes. We used real time RT-PCR to examine the expression of each FRO gene in different tissues and in response to iron limitation. FRO3 is expressed at high levels in leaves and roots of seedlings and expression of FRO3 is induced by iron-deficiency. FRO3-GUS transgenic plants reveal that the FRO3 promoter is primarily active in the vascular tissue of the plant and *FRO3-GFP* stable transgenic lines show that FRO3 is localized at the plasma membrane. Analysis of a FRO3-KO line shows that iron accumulation is altered in this line as compared to wild type as is the expression of a variety of genes involved in iron uptake, localization and storage. Taken together, our results show that FRO3 functions in maintenance of iron homeostasis and suggest that FRO3 functions in long-distance transport of iron.

335 Analysis of the Complex *BIO3 / BIO1* Locus of *Arabidopsis*

Rosanna Muralla[1], Colleen Sweeney[1], Elve Chen[2], Libuse Brachova[2], Basil Nikolau[2], David Meinke[1]

[1]Oklahoma State University, Stillwater, OK, USA, [2]Iowa State University, Ames, IA, USA

The biosynthesis and utilization of biotin in plants has been elucidated in part through the analysis of two auxotrophic mutants of *Arabidopsis* (*bio1* and *bio2*) first identified 15-20 years ago using a forward genetic screen for embryo-defective mutants rescued by growth of arrested embryos on enriched media. We demonstrate here through reverse genetics that the *bio3* mutant, which is disrupted in a region predicted to encode an enzyme that functions in an intermediate step in the pathway, has a phenotype similar to that of the *bio1* and *bio2* mutants. The surprising discovery is that the *BIO3* and *BIO1* loci are positioned adjacent to each other on the chromosome, in the same orientation as found in many bacterial and fungal species, and that differential splicing results in the production of two types of transcripts, one with the potential to encode two separate proteins, and the other capable of producing a fusion protein that catalyzes two different steps in the pathway. The existence of the fusion protein in plant extracts is being tested using antibodies directed against each of the monocistronic gene products produced in *E. coli*. The results obtained to date have provided important clues to the genomic organization of biotin biosynthetic genes in *Arabidopsis*, the intracellular localization of biotin synthesis, and the evolutionary remnants of a prokaryotic operon in a flowering plant.

336 Characterization of GDU1 and GDU1-Like Genes Involved in the Regulation of Amino Acid Metabolism and Transport

Rejane Pratelli[2], Wolf Frommer[1], Guillaume Pilot[2]

[1]Carnegie Institution, 260 Panama Street, Stanford, CA 94305-4101 USA, [2]IZMB Universitaet Bonn, Kirschallee 1, 53115 Bonn, Germany

The GDU1 (Glutamine Dumper 1) gene was identified by the study of an activation tagged mutant from Arabidopsis (gdu1-1D). This gene encodes an uncharacterized, 158 amino acid-long protein, expressed in the vascular tissues, in both phloem and xylem parenchyma cells. GDU1 protein contains a single transmembrane spanning region and seems to be targeted to the plasma membrane and the membrane of yet undefined vesicles (1). Arabidopsis genome encodes five proteins sharing between 32 and 76% overall similarity with GDU1 and showing strong sequence conservations in two domains that are specific of this family of plant proteins. The promoters of the GDU1-like genes are active in various organs of the plant, each gene showing a distinct expression pattern. This suggests that the GDU genes present similar functional properties but have different roles due in part to their specific expression pattern and possibly to the sequence differences they display outside of the two conserved domains.

The activation-tagged gdu1-1D mutant over-expresses GDU1. These plants are smaller than the wild type, secrete glutamine and sodium at the hydathodes and display necrosis spots on the older leaves. The leaf content of each of the free amino acids is increased in the mutant compared to the wild type, resulting in a doubling of the overall amount of free amino acids. The concentration of amino acids in the phloem and the xylem saps is also increased twofold in the mutant. It is thought that the glutamine secreted from the hydathodes originates from the xylem sap, as glutamine constitutes about 90% of xylem sap amino acids. The hydathode tissues from the mutant would thus be deficient in reabsorbing the xylem amino acids. Wild type plants are unable to grow on media containing high concentrations of several amino acids (e.g. Met, Thr, Phe, Tyr) because of the feedback inhibition of biosynthetic pathways induced by these amino acids. Interestingly, the gdu1-1D mutant is unaffected by these high concentrations, reminiscent of the phenotype of the pig1-1 mutant (2), which is supposed to be altered in the regulation of amino acid metabolism. These data suggest that the transport and the metabolism of amino acids are altered in the gdu1-1D mutant.

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337 Interaction-dependent localization of flavonoid enzymes in *Arabidopsis*

Melissa Ramirez, Brenda S. Winkel
Virginia Polytechnic Institute and State University

As early as the 1940's, the idea of multienzyme complexes began surfacing as a way the cell might organize enzymes to enhance the efficiency of metabolism. It has now become evident that macromolecular interaction is a fundamental aspect of cellular biochemistry. The Winkel laboratory uses the flavonoid pathway of Arabidopsis as a model to understand the assembly and regulation of enzyme complexes. This pathway is a well-characterized specialized plant metabolic system, the products of which comprise a diverse set of compounds that are critical for plant survival and reproduction. While the central steps of flavonoid biosynthesis are well detailed, a full understanding of the biochemistry of the pathway is complicated by the fact that many of the enzymes can utilize multiple substrates and may also physically interact with each other.

Until recently, it was believed that flavonoid biosynthesis occurred exclusively in the cytoplasm and that the products were then transported to various sites of action within the cell. However, new evidence indicates the presence of at least two flavonoid enzymes in the nucleus, suggesting that the synthesis of nuclear flavonoids may occur in situ. Additional studies are needed to explore the possibility that subcellular localization of flavonoid enzymes is affected by or dependent upon specific protein-protein interactions and that this localization determines the types and cellular locations of end products that are produced in response to diverse biotic and environmental cues.

The study presented here begins to dissect the molecular basis of the observed dual localization of chalcone synthase (CHS) and chalcone isomerase (CHI), the first two enzymes of the flavonoid pathway. Epi-fluorescence and confocal laser scanning microscopy are being used to examine the localization of these enzymes expressed as fusions to green fluorescent protein (GFP) in protoplasts and stably-transformed plants. Analyses are being performed in both wild type cells and mutants that are depleted in various flavonoid enzymes in order to explore the possible effects of specific protein-protein interactions on this localization. Site-directed mutagenesis is being used in parallel experiments to test the functionality of a putative nuclear localization signal in CHS, which overlaps with the predicted CHI interaction interface. This work represents an essential step in elucidating the mechanisms that organize related metabolic enzymes within the crowded environment of the cell interior.

338 FAC1-Directed Herbicide Toxicity Correlates With Expanded Adenine Nucleotide Pools

Richard Sabina
Medical College of Wisconsin

EMBRYONIC FACTOR 1 (FAC1) is an early expressed plant gene and knockout lines reveal that it is essential for the zygote to embryo transition in Arabidopsis thaliana (Plant J 42:743,2005). FAC1 encodes an AMP deaminase (AMPD), which is also the intracellular target for a class of modified nucleosides produced by fungal pathogens that are converted in plant cells to transition-state inhibitors (monophosphates) of this enzyme (Plant Physiol 114:119,1997;Bioorg Med Chem Lett 9:1985,1999). Exposure to these herbicides results in a rapid 2-3 fold increase in ATP levels, but reportedly not ADP or AMP, and eventual cessation of seedling growth with paling and necrosis at the apical meristem. The x-ray crystal structure of Arabidopsis FAC1 bound to a transition-state analog has provided a glimpse of the complete active site (J Biol Chem 281:In Press,2006). However, the mechanistic basis for lethality associated with a genetic or herbicide-induced limitation in FAC1 catalytic activity is unknown. OBJECTIVE: Explore the underlying mechanisms for FAC1-directed toxicity by considering the immediate metabolic consequences of an inability to deaminate AMP in plant cells. APPROACH: Monitor relative growth and quantify intracellular adenine nucleotides (AXP) and IMP in treated and untreated control Arabidopsis seedlings following systemic exposure to sublethal and lethal doses of deaminoformycin (DF) and attempt rescue with purine nucleosides and bases. METHODS: 5 to 7 d.o. seedlings were placed on M&S salt + 1% sucrose agar in 24-well plates with and without DF (300 nM or 22 uM) and/or a purine nucleoside or base (0.5-1 mM). Plants were grown under long-day conditions (16 h light/8 h dark) for 7-9 days, roots were excised and the remaining tissue was weighed and ground to a powder under liquid nitrogen. Ice-cold 10% (w/v) TCA was added and the frozen tissue was homogenized on ice for 2 min with a motorized pestel. The homogenate was centrifuged at 14,000Xg for 2 min at 4C and the supernatant was neutralized with an equal volume of 0.5 M tri-n-octylamine in freon. All samples were frozen immediately in dry ice and stored at -80C. Metabolites were separated by anion-exchange HPLC. RESULTS: DF inhibits plant growth and wet weight is inversely correlated with the levels of ALL adenine nucleotides and the AMP/IMP ratio, an in vivo index of AMPD activity. Downstream catabolites do not rescue seedlings from a lethal dose of DF. The correlations between decreased AMPD activity, increased AXP pools, and reduced seedling growth point to mechanisms related to upstream effects of FAC1 inhibition. This may involve hormonal imbalance due to increased cytokinin synthesis or disruption of 14-3-3 protein function by AMP.

This work was supported by a grant from the Research Affairs Committee (RAC) at the Medical College of Wisconsin and through a cooperative agreement with Bayer CropScience GmbH, Frankfurt am Main, Germany.

339 Exploring of Arabidopsis non-host resistance via high resolution profiling of plant secondary metabolites

Dierk Scheel, Christoph Boettcher, Edda von Roepenack-Lahaye, Stephan Clemens
Leibniz Institute of Plant Biochemistry, Halle, Germany

We developed a platform for the highly sensitive profiling of mostly secondary metabolites, employing capillary liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (CapLC-ESI-QTOF-MS). This approach achieves a very good coverage of Arabidopsis secondary metabolism. A recent compilation listed six biosynthetic classes: nitrogen-containing compounds, phenylpropanoids, benzenoids, polyketides such as flavonoids, terpenes and fatty acid derivatives. Metabolites of five of these classes can clearly be detected by CapLC-ESI-QTOF-MS. Furthermore, in-source fragmentation and targeted tandem MS analysis allow to obtain structural information on unknown compounds. This is of paramount importance given, for instance, the conservatively estimated 5000 metabolites in Arabidopsis thaliana of which maybe only 500 are annotated today. Databases for LC-MS spectra and for known and "theoretically" occurring compounds in the Brassicaceae help in structural elucidation and in cataloguing the Arabidopsis metabolome. A systematic evaluation of matrix effects has shown that the good separation achieved allows reproducible quantification. The platform and its applicability as a general method for biochemical phenotyping of Arabidopsis mutants will be presented. In particular, we show here profiling data of Arabidopsis mutants with altered non-host resistance against fungal and oomycete pathogens.

340 Arabidopsis Sucrose Transporter AtSUC9: High Affinity Sucrose Transport, Intragenic Control of Expression and Comparative Substrate Specificity

Alicia Sivitz, Anke Reinders, Meghan Johnson
University of Minnesota

Transport of sucrose across membranes is controlled by sucrose transporter proteins (SUTs or SUCs). Plants have small gene families of SUTs; the Arabidopsis genome contains seven SUT genes. Here, the Arabidopsis thaliana sucrose transporter AtSUC9 (At5g06170) was expressed in Xenopus oocytes and revealed to have an ultra-high affinity for sucrose (K0.5 = 0.066 +/- 0.025 mM) compared to other plant sucrose transporters. AtSUC9 also showed low specificity and transported a wide range of glucosides including helicin, salicin, arbutin, maltose, fraxin, esculin, turanose, and alpha-methyl D glucose. AtSUC9 substrate specificity was found to be similar to AtSUC2 (At1g22710). The ability of AtSUC9, AtSUC2, HvSUT1 (from barley) and ShSUT1 (from sugarcane) to transport a variety of substrates was compared, and the results indicate that Type I and Type II sucrose transporters have different substrate specificities. AtSUC9 expression was found in sink tissues throughout the shoots and in flowers. AtSUC9 expression was dependent on intragenic sequence, which was also true for AtSUC1 (At1g71880), but not AtSUC2. In summary, the novel transporter AtSUC9 has a much higher affinity for sucrose than any other plant sucrose transporters, indicating that AtSUC9 is uniquely suited to function in maintaining very low sucrose concentrations in the wall space around shoot sink cells.

341 Genetic Dissection of Histidine Biosynthesis in *Arabidopsis*

Colleen Sweeney[1], Rosanna Muralla[1], Devin Camenares[2], Patricia Nugent[2], Christopher DiFraia[2], Asya Stepansky[2], Thomas Leustek[2], David Meinke[1]

[1]Oklahoma State University, Stillwater, OK, USA, [2]Rutgers University, New Brunswick, NJ, USA

The biosynthesis of histidine in microorganisms, long studied through the isolation and characterization of auxotrophic mutants, has emerged as a paradigm for the regulation of metabolism and gene expression. Much less is known about histidine biosynthesis in flowering plants. One limiting factor has been the absence of large collections of informative auxotrophs. We describe here the results of a systematic screen for histidine auxotrophs of *Arabidopsis thaliana*. Nine insertion mutants disrupted in four different biosynthetic genes (*HISN2*, *HISN3*, *HISN4*, *HISN6A*) were identified through a combination of forward and reverse genetics and were shown to exhibit an embryo-defective (*emb*) phenotype that could be rescued by watering heterozygous plants with histidine. Male transmission of the mutant allele was in several cases reduced. Another mutant blocked in the final step of the pathway (*hisn8*) and a double mutant altered in the redundant first step (*hisn1a, hisn1b*) exhibited an ovule abortion (*ova*) phenotype in heterozygotes. Homozygous mutant plants and callus tissue produced from rescued seeds appeared normal when grown in the presence of histidine but typically senesced after continued growth in the absence of histidine. These mutants document the importance of histidine biosynthesis for plant growth and development, provide valuable insights into amino acid transport and source-sink relationships during seed development, and represent a significant addition to the limited collection of well-characterized auxotrophs in flowering plants.

342 Proteomic Analysis of the Flavonoid Biosynthetic Machinery in *Arabidopsis thaliana*

Nileshwari Vaghela[1], Iuliana Lazar[1, 2], Brenda S. Winkel[1]

[1]Department of Biological Sciences, Virginia Tech, [2]Virginia Bioinformatics Institute (VBI), Virginia Tech

Work on a wide variety of metabolic pathways indicates that these systems are often, if not always, organized as multienzyme complexes. Enzyme complexes have the potential to increase catalytic efficiency and provide unique mechanisms for the regulation of cellular metabolism. The flavonoid biosynthetic pathway of Arabidopsis is an excellent model for studying the organization, localization, and regulation of enzyme complexes at the cellular level [1]. Flavonoids are specialized metabolites that perform many important physiological roles in plants. Protein interactions among several key flavonoid enzymes have been described [2]. Moreover, at least two of the flavonoid enzymes have a dual cytoplasmic/nuclear localization [3]. These results indicate that flavonoid enzymes assemble into one or more distinct complexes at different intracellular locations.

The current study integrates a new technology, mass spectrometry, with well-established affinity chromatography methods to further characterize the organization and composition of the *Arabidopsis* flavonoid enzyme complex. Two key flavonoid enzymes, chalcone synthase (CHS) and chalcone isomerase (CHI), are being used in these experiments to detect interacting enzymes. Recombinant thioredoxin (TRX), TRX-CHS, or TRX-CHI is produced in E. coli [4,5], then purified by metal affinity chromatography, and covalently coupled to an activated resin, Affi-Gel 10 [2]. Extracts prepared from 5-day-old wild type or CHS- or CHI-deficient lines of *Arabidopsis* are then passed over the column and the bound proteins are eluted with SDS. This eluate is then subjected to a liquid chromatography (LC) - mass spectrometry (MS) protocol developed for the analysis of complex peptide mixtures [6]. An Agilent LC system coupled with an LTQ-MS instrument (Thermo Finnigan, San Jose, CA) is being used for this purpose. Data analysis is being performed with BioWorks II software. This project offers a new prospect for confirming previously-described interactions among flavonoid enzymes and for identifying novel interacting proteins, thereby enhancing our understanding of the flavonoid enzyme complex as a whole.

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343 A Yeast-2-Hybrid Assay Reveals that Chalcone Synthase and Chalcone Isomerase Selectively Bind Proteins Encoded by Non-Flavonoid Related Genes

Jonathan Watkinson, Brenda Winkel
Virginia Tech

The biosynthetic enzymes of the flavonoid pathway are generally believed to form a complex or metabolon. This multi-enzyme complex allows for tight regulation over the synthesis of flavonoid end-products and prevents the build-up of toxic intermediates by channeling them from one enzyme in the pathway to the next. Originally, the complex was believed to be located exclusively at the cytoplasmic face of the endoplasmic reticulum with flavonoid 3' hydroxylase as the membrane anchor. However, there is now evidence that nuclear localization of chalcone synthase (CHS), chalcone isomerase (CHI) and perhaps other flavonoid enzymes are also targeted to nuclei and that this localization may be dynamic, changing in response to external stimuli and possibly, developmental cues.

We have undertaken a yeast-2-hybrid screen to search for binding partners of flavonoid enzymes in an effort to determine whether other factors can affect localization of the metabolon. CHS, CHI, and flavanone 3 hydroxylase (F3H) were previously cloned into the yeast pBI880 DNA binding domain bait vector. An Arabidopsis, cDNA, activation domain (prey) library in pBI771 was used to transform HF7c yeast cells harboring one of the three bait vectors. The transformed cells were plated onto selective synthetic dextrose medium lacking histidine and supplemented with 3-aminotriazole. Positives were confirmed by plating on medium lacking uracil as well as histidine and by confirming that no activation of the reporter gene took place in the absence of bait. Plasmids were isolated from these colonies and the sequences of the inserts determined. From the CHS screen eight positive clones have been isolated. The encoded proteins include ribosomal proteins, a GA responsive protein, a cytosolic amino peptidase and a putative FYVE domain type protein. Knockout plants for the cytosolic amino peptidase and putative FYVE domain protein are being analyzed. Interaction between CHS and these proteins is also being characterized using surface plasmon resonance refractometery. The screen for CHI generated four positives of which two have been eliminated and 2 are undergoing further analysis. Screening of F3H did not generate any positive hits in the initial experiment.

344 Closely related Arabidopsis thaliana R2R3-MYB transcription factors act as distinct flavonol-specific regulators of phenylpropanoid biosynthesis

Ralf Stracke, Frank Mehrtens, Bernd Weisshaar
Bielefeld University

The Arabidopsis thaliana R2R3-MYB transcription factor MYB12 was identified as a flavonol-specific activator of flavonoid biosynthesis. A high degree of functional similarity between MYB12 and the structurally closely related factor P from Zea mays was revealed by transient expression in A. thaliana protoplasts. Both displayed similar target gene specificity, and both activated the target gene promoter only in presence of a functional MYB recognition element (MRE). The genes encoding the flavonoid biosynthesis enzymes CHS, CFI, F3H and FLS were identified as target genes. A tight linkage between the expression level of functional MYB12 and the flavonol content of young seedlings was observed by HPLC analyses of myb12 mutants and MYB12 overexpression plants. qRT-PCR using seedlings of these mutant plants showed MYB12 to be a transcriptional regulator of CHS and FLS in planta. These enzymes are essential for flavonol biosynthesis and katalyze key branch point steps in the pathway. Transient expression of the closely related A. thaliana R2R3-MYB factors MYB11 and MYB111 (together with MYB12 defining the subgroup 7 of the R2R3-MYB gene family) showed similar target gene specificity. Analyses of myb11 and myb111 mutant plants revealed impact of the corresponding genes on flavonol biosynthesis.

345 Physiological function of HMA2 and HMA4 in Arabidopsis thaliana

Edwin Wong, Renee Jarvis, Christopher Cobbett
The University of Melbourne

Type-1_B P-type ATPases utilize ATP-hydrolysis to transport heavy metals across various biological membranes. Current study in our lab is focusing on the characterization of two of the eight P-1_B transporters in Arabidopsis thaliana, HMA2 and HMA4. Genetic analysis has shown HMA2 and HMA4 are essential for zinc homeostasis: a hma2;hma4 double mutant is severely zinc deficient (1). Symptoms include chlorosis, stunting and failure to develop pollen, and these phenotypes are reversible by the application of exogenous zinc. Promoter-GUS reporter constructs have shown HMA2 and HMA4 have parallel expression patterns in vascular tissues and in developing anthers. Expression of the HMA proteins in yeast has effects on cadmium resistance and zinc dependence. As divalent cation transporters, HMA2 and HMA4 may function in Cd translocation and detoxification in addition to maintaining Zn homeostasis. In plants, phytochelatins are a major determinant of Cd detoxification. Upon exposure to heavy metals, these heavy metal-binding peptides are synthesized enzymatically from glutathione by CAD1-encoded phytochelatin synthase. cad1 mutants are phytochelatin deficient and hypersensitive to Cd (2). We have created multiple cad1;hma mutant lines. These are being analysed for altered Cd-sensitivity and Cd uptake and translocation. Both HMA2 and HMA4 have potential metal-binding motifs. For example, in the N-terminal region, HMA2 has GICC instead of the more common metal binding motif GMxCxxC and, in an extended C-terminal domain there are multiple di-Cysteines and a poly-His region. We are expressing various mutant derivatives of HMA2 in the hma2;hma4 double mutant in an attempt to understand the in planta physiological significance of these motifs.

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346 Identification and Characterization of Pyruvate Decarboxylase *(pdc)* Gene Family Members in *Arabidopsis*

Songqing Ye, Jerry Cohen
Graduate Program in Plant Biology, Department of Horticultural Science, and Microbial and Plant Genomics Institute, University of Minnesota, Saint Paul, MN 55108 USA

Pyruvate decarboxylase (PDC, EC 4.1.1.1) converts, by decarboxylation, pruvate into acetaldehyde and then alcohol dehydrogenase (ADH, EC 1.1.1.1) reduces acetaldehyde into ethanol. This set of reactions shunts the main glycolytic pathway into ethanol fermentation, instead of entry into the tricarboxylic acid cycle, so it is very important for plants as they respond to anaerobic stress. By searching the Arabidopsis database, we found that there are four genes having the high sequence similarity to the *pdc* genes reported from bacteria. The 4 putative *ipdc*/*pdc* genes are: *pdc4* (At5g01320), *pdc3* (At5g01330), *pdc2* (At5g54960), and *pdc1* (At4g33070). A phylogenetic tree, based on the full amino acid sequences of known pdc genes was constructed using a neighbor-joining method. All plant putative pdc genes were shown to be conserved and clustered together. Pdc1 and pdc2 share high sequence similarity (82% identity). Both pdc3 and pdc4 are located on chromosome 5, separated by 1.6kb, and show 92% identity. We have cloned these genes from cDNA (ABRC) or a cDNA library and expressed and purified the recombinant proteins by the His-tag technique.

We found that AtPDC2 is functional PDC according to its measured biochemical activity. The optimal pH for the recombinant protein was 6.2 and the Km was 3.5 mM. Typically, PDCs require the cofactors Mg^{2+} and thiamin pyrophosphate (TPP). We found 0.5 mM TPP and 5 mM Mg^{2+} resulted in the highest activity, however, the plant enzyme still showed around 40% of its activity when supplied with only a single cofactor or without any cofactor, compared with an optimal combination of both cofactors. In bacteria and fungi, PDCs have bi-functional activities and always show some IPDC (indole pyruvate decarboxlase) activity as well. However, we did not find any IPDC activity for AtPDC2 suggesting that uniquely this plant enzyme is mono-functional. Also, AtPDC1 and AtPDC3 have not measurable PDC activity. This work was supported, in part, by a grant from the U.S. Department of Energy.

347 Genetic Mechanisms of Glucosinolates Hydrolysis in Crucifers

Zhiyong Zhang, James Ober, Daniel Kliebenstein
University of California-Davis

Glucosinolates are sulfur-rich plant secondary metabolites present in all cruciferous plants that require hydrolysis for activation. Their breakdown products have a wide range of biological activities in plant-herbivore and plant-pathogen interactions and anti-carcinogenic properties that are determined by the resulting chemical structure. In *Arabidopsis* and *Brassica*, hydrolysis by the enzyme, myrosinase, produces the bioactive nitriles, epithionitriles or isothiocyanates depending upon the plants genotype and the glucosinolates structure. Two QTL, *ESP* and *ESM1*, function to determine formation of epithionitriles and nitriles. We have cloned both QTL and they have opposing functions. *ESP* directs nitrile formation while *ESM1* inhibits nitrile formation. Both proteins have been shown to interact with myrosinase in other species suggesting that the myrosinase protein complex functions to control the structural outcome of glucosinolate hydrolysis. We have extended this work to show that *ESP* and *ESM1* control the *in planta* formation of Indole-3-acetonitrile, a potential auxin precursor. In addition to the biochemical function, we are studying the downstream impact on *Arabidopsis*/Insect interactions. The glucosinolate hydrolysis profile change towards isothiocyanate formation controlled by *ESM1* is associated with resistance to *Trichoplusia ni*. We have recently identified associated variation within *Brassica* vegetables that could allow for potential improvement of human nutrition. Work on associating this phenotypic variation with genetic variation within *ESP* and *ESM1* will be presented. We have recently identified associated variation within vegetables that could allow for potential improvement of human nutrition.

The myrosinase protein complex is comprised of a large number of proteins. A number of these proteins are *ESP* or *ESM1* homologues. Interestingly, the *ESP* and *ESM1* homologues are interspersed in the same two gene clusters on Chromosome 1, and another is on Chromosome 3. Thus, suggesting that they are undergoing parallel evolution. These homologues show high similarity with *ESM1* or *ESP* in both gene structure and protein features. We are currently using T-DNA Knockouts, 35S-overexpression and biochemistry to test if these homologues also control structural specificity during glucosinolate hydrolysis. Preliminary genomic analysis will be presented.

Department of Plant Sciences, University of California, Davis, CA 95616.
To whom correspondence should be addressed: kliebenstein@ucdavis.edu

348 Isolation for mutants of higher accumulated elements and pigments from rice FOX lines

Youichi Kondou[1], Mika Kawashima [1], Takanari Ichikawa [1], Akie Ishikawa [1], Hirohiko Hirochika [2], Minami Matsui [1]
[1]PSC, RIKEN, [2]National Institute of Agrobiological Sciences

FOX hunting system is novel gain-of-function technique. In this system, random over-expression of a normalized plant's full-length cDNA library cause dominant gain-of-function mutations and it is expected that plant acquire useful phenotype. We are establishing rice FOX lines using 13,000 non-redundant rice full-length cDNAs to introduce them into *Arabidopsis* plants. At present, we are screening these lines with various trait for exploration of useful rice genes. In this study, we will introduce screening strategy for isolation of mutants of higher accumulated elements and pigments (Elements and Pigments) in rosette leaves as examples of useful phenotype. We already isolated some mutants by pre-screening in T1 generation from about 3,000 lines.

This research is supported by the Special coordination funds for Promoting Science and Technology entitled Rapid identification of useful traits using rice full-length cDNAs.

349 Complexity Reduction of Polymorphic Sequences (CRoPS): A Novel Approach For High Throughput Polymorphism Discovery

Michiel van Eijk, Nathalie van Orsouw, Jan van Oeveren, Rene Hogers, Antoine Janssen, Sandor Snoeijers, Esther Verstege

Keygene N.V.

Discovery of polymorphisms, including single nucleotide polymorphisms (SNP) and simple sequence repeats (SSR), in low polymorphic species is still a challenging and costly endeavor, despite widespread availability of capillary electrophoresis sequence technology. We present a novel approach for polymorphism discovery (CRoPS) in plants by combining the power of reproducible genome complexity reduction of AFLP with recently developed breakthrough sequencing by synthesis (pyrosequencing) technology of 454 Life Sciences (Margulies et al., 2005. Nature 437, 376-380), commerialized as Genome Sequencer 20 (GS 20) by Roche Diagnostics.

The principle or CRoPS is that tagged complexity-reduced libraries of two or more genetically diverse samples are prepared by AFLP and sequenced at 5-10-fold redundancy in microfabricated high-density picolitre reactions. A typical sequence-run yields over 200,000 sequence reads with a median length of 100 bases. Resulting sequences are clustered and sequence contigs inspected for sequence differences using bio-informatics tools. Rigorous quality measures are applied to separate sequence errors from true polymorphisms, based on redundant sequencing, sample origin information and allele frequencies. Using CRoPS, low cost polymorphism discovery will become affordable in organisms with low levels of germplasm polymorphism and / or highly repeated genomes, which includes many crop species. CRoPS results of SNP and SSR mining and validation in pepper and maize will be presented. Widespread availability of SNP markers will expedite the use of novel high-throughput SNP detection platforms to drive down the cost of marker-assisted breeding approaches. Alternative applications of the CRoPS technology currently in development are sequence-based transcript profiling and genotyping.

350 Fine mapping of flowering Arabidopsis QTLs of small effect

Eduardo Sanchez-Bermejo, Belen Mendez-Vigo, Jose Martinez-Zapater, Carlos Alonso-Blanco

Departamento de Genetica Molecular de Plantas, Centro Nacional de Biotecnologia, Consejo Superior de Investigaciones Cientificas (CNB-CSIC), Darwin 3, Cantoblanco, Madrid-28049, Spain.

Wild genotypes of Arabidopsis collected from different natural populations differ strongly in their flowering time behaviour and, presumably, this variation is involved in the adaptation to different environments (Koornneef et al. 2004). Quantitative genetic analyses of the naturally occurring variation for flowering initiation in Arabidopsis have identified five major effect QTLs that have been already isolated, and a higher number of small effect ones (Alonso-Blanco et al., 2005). To understand the genetic and molecular bases of this variation we have systematically developed single introgression lines that carry either early or late flowering alleles from 5 different wild accessions in the reference Landsberg erecta (Ler) genetic background. Based on those introgression lines, we have identified 14 different genomic regions affecting flowering time located in all five chromosomes, which we have named as Flowering Arabidopsis QTLs (FAQ). Currently, we have begun the fine mapping of several FAQ regions identified in several crosses as carrying alleles of small effect under long-day photoperiod, such as FAQ2 located on top of chromosome 1 and FAQ3 located on chromosome 3. For that, we have developed new sets of Ler background introgression lines where each line carries a smaller introgression segment of different size, but the various introgressions of the set overlap like a stair (Komproglou et al., 2002). Using this approach that we refer to as stairs of mini-introgression lines, we have found that FAQ2 is a complex QTL consisting of at least two closely linked loci, while FAQ3 seems to be a single locus.

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351 Potent Induction of flowering by elevated growth temperature in Arabidopsis thaliana

Sureshkumar Balasubramanian[1], Sridevi Sureshkumar[1], Janne Lempe[1], Detlef Weigel[1, 2]

[1]Max-Planck Institute for Developmental Biology, [2]Salk Institute, La Jolla, USA

The transition from the vegetative to reproductive development in plants is influenced by endogenous developmental cues as well as environmental signals. The major environmental factors that modulate floral transition include photoperiod, light quality, vernalization (exposure to winter conditions) and growth temperature. In addition biotic and abiotic stress have also been implicated in floral transition. Uncoupling and studying the specific effects of each of these variables have been challenging. While the photoperiod and vernalization pathways have been explored in greater detail, the molecular genetic basis of the effects other environmental variables on flowering remains enigmatic. We have addressed the effects of growth temperature, using an assay that allows us to study the specific effects of temperature on floral transition, through a combinatorial analysis of mutant and natural genetic variants. We report a strong induction of flowering in short day grown plants at elevated growth temperature (27ºC as opposed to the commonly used 23ºC). The floral induction is as strong as plant growth long day conditions at lower temperatures (16ºC LD) and there is extensive natural variation in this response. Analysis of natural variants and flowering time mutants show FLOWERING LOCUS C (FLC) to be a potent suppressor of thermal induction similar to its effects on photoperiodic induction. However suppression of flowering at lower temperatures (23ºC) is not brought about exclusively through FLC. We have performed QTL mapping to uncover additional loci involved in thermal response. Using recombinant inbred lines derived from parental lines that differ in thermo sensitivity, we show a quantitative trait locus (QTL) for thermo sensitivity maps to the floral repressor FLOWERING LOCUS M (FLM) indicating the thermal inductive pathway acts in the same genetic cascade that of FLM. While flc mutants are more sensitive to temperature consistent with the repressive effect of FLC, the flm mutants are less sensitive to temperature indicating the thermal induction acts in the same genetic cascade to that of FLM. Thermal induction is independent of CONSTANS and is integrated at the level of floral pathway integrators. Microarray analysis reveals the genomic response to thermal and photoperiodic floral induction differs and factors involved in alternative splicing are enriched specifically during thermal shift. This enrichment appears to be associated with changes in splicing patterns of at least some of the flowering time regulators.

352 Natural Variation in the Arabidopsis Ionome

Ivan Baxter[1], Ana Rus[1], Brett Lahner[1], Elena Yakubova[1], Monica Borghi[1], Owen Hoekenga[2], David Salt[1]

[1]Purdue University, [2]Boyce Thompson Institute for Plant Research, Cornell University

Uncovering the genes that underpin mineral ion homeostasis in plants is a critical first step towards understanding the biochemical networks that regulate the plants ionome. The natural accessions of *Arabidopsis thaliana* provide a rich source of genetic diversity that leads to phenotypic differences. Additionally, changes in the ionome in natural accession may represent adaptations to the local growth environment. To identify genes responsible for variation in mineral ion homeostasis among accessions, we measured the levels of Li, B, Na, Mg, P, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo and Cd, in the shoots of 96 different accessions. The majority of accessions assayed showed significant differences in the shoot accumulation of at least one element when compared with the reference accession Col-0. We also analyzed the seed ionome of 12 of the accessions. Interestingly, there was very little correspondence between the elemental differences in the shoots and seeds of the same accession. To determine the degree of overlap between the loci which control the ionome in different accessions, we measured the elemental accumulation in the leaves of three Recombinant Inbred Line populations: Col-4 x Ler-0, Bay-0 x Sha, and Cvi-1x Ler-2. Over 100 QTLs were identified in the populations, including at least one for each element analyzed. More than 65% of the loci identified were unambiguously unique to a single population. Analysis of the Cvi-1x Ler-2 population under Fe deficient growth conditions, revealed an additional 18 QTLs, only four of which were found in the original, Fe sufficient, growth condition. We have identified novel alleles of three genes which alter the accumulation of Na, Mo and Co in different Arabidopsis accessions.

353 Natural Variation Studies For Circadian Clock Input Components In Arabidopsis thaliana

Eleni Boikoglou[1, 2], Amanda Davis[1], Ferenc Nagy[2], Seth Davis[1]

[1]Max Planck Institute for Plant Breeding Research, Koeln, 50829, Germany, [2]Institute of Plant Biology, Biological Research Center, H-6701, Szeged, Hungary

The circadian clock is an internal mechanism that regulates various processes in the plant life cycle. The clock is entrained by environmental cues, such as light and temperature. Although light input has extensively been studied, temperature's role as a clock input signal is as yet unraveled. Recently, studies on circadian mutants have shown that temperature cycles are capable to entrain the clock. Our interest is to dissect the genes that function in this thermo-sensory pathway. Presumably, the genetic architecture of thermo-perception will be similar to, but different from, photo-perception. In this study, natural variation is exploited with respect of thermal entrainment of the circadian clock. Arabidopsis thaliana accessions and Recombinant Inbred Lines (RILS) were transformed using CCR2::LUC reporter. The CCR2 promoter was chosen as its expression is under clock control, and moreover, is thermally regulated. Clock characteristic-traits such as period, phase, and amplitude will be assessed by measuring bioluminescent rhythms of CCR2 transcriptional outputs. Our aim is to identify, map, and characterize temperature-specific genetic components responsible for regulation of the circadian clock. Preliminary results of various lines have revealed that there is low variation within the RILS and high variation amongst them. Thus, it is feasible the scoring of the clock traits will contribute to QTL identification. The identification of these genetic components will lead towards an exciting entry in the temperature entrainment of the circadian clock.

354 Prevalence and mechanisms of F1 incompatibility in Arabidopsis thaliana

Kirsten Bomblies, Janne Lempe, Christa Lanz, Norman Warthmann, Detlef Weigel

Max Planck Institute for Developmental Biology

Postzygotic reproductive incompatibility, that is, the sterility or inviability of hybrids, dramatically illustrates breakdown of gene coordination within a genome and is likely important in speciation. We show here that post-zygotic incompatibilty exists among wild strains of Arabidopsis thaliana. Among over 300 hybrid combinations we identified five F1 hybrids that show environmentally sensitive morphological defects, ranging in severity from leaf chlorosis and twisting, to dwarfism, loss of apical dominance, severe leaf defects and growth arrest. The genetic interactions conform to predictions of the Dobzhansky-Muller model for post-zygotic incompatibility involving two dominant loci. All five hybrids are specific; crosses with other ecotypes did not produce abnormalities. Furthermore, hybrid combinations among the parents of these hybrids show that different genes or alleles underlie the F1 phenotypes. Using micro-arrays, histological and other molecular approaches, we found that these hybrids mount autoimmune responses in the absence of pathogen challenge. Consistent with this, one of two causal regions fine-mapped in one hybrid contains two polymorphic TIR-NBS-LRR class resistance (R) genes. We are now testing candidate genes using artificial micro-RNAs and are mapping the causal loci for the remaining four hybrids. Intriguingly, our Arabidopsis hybrid incompatibility phenotypes (including temperature sensitivity) are remarkably similar to a common F1 hybrid syndrome, hybrid necrosis or weakness, which occurs in a wide variety of other plant species. Based on our data, we hypothesize that rapidly-evolving R genes evolve aberrant interactions with gene variants present in conspecific individuals, resulting in hybrid incompatibility at detectable frequency in Arabidopsis and other plant species. This parallels findings in animals that implicate rapidly evolving genes in reproductive isolation and has important implications for plant evolution and speciation.

355 Phylogenetic Analysis of Arabidopsis PcG Protein EMF2

Lingjing Chen, Renee Sung
Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

Arabidopsis Polycomb group (PcG) genes regulate development via repression of distinct MADS-box genes. Arabidopsis proteins EMBRYONIC FLOWER2 (EMF2), VERNALIZATION2 (VRN2), and FERTILIZATION INDEPENDENT ENDOSPERM2 (FIS2) share similar VEF domain to Drosophila PcG protein Su(z)12. Here we report that VEF-domain-containing genes extensively exist in angiosperm, while being a small gene gamily in each species. Three families were recognized based on their domain organization, with EMF2 family that includes EMF2 and VRN2 as the biggest family. Widespread distribution of EMF2 homologs in angiosperm indicates that their early version occurred before dicots and monocots diverged. Global homology strongly suggests that VRN2 subfamily is an abbreviated version from an ancestral EMF2. The members of VRN2 subfamily occur in Salicaceae, Leguminosae, Brassicaceae, and Solanaceae, all members of eudicots, suggesting that they split from EMF2 lineage at a later stage in angiosperm evolution. The INDEL sequences of current members of EMF2 family showed a closest relationship between Nuphar and basal monocot Acorus, as well as Yucca and Asparagus, supporting a Nyphaeaceae-related origin of Monocots. The unique domain organization in FIS2 and VEF-L36 families suggests that they belong to other lineages in VEF superfamily. Intragenic sequence duplication, deletion/insertion, and intergenic exon shuffling found in these three families should account for their functional innovation.

356 Genetic Mechanisms of Seasonal Maternal Effects on Germination Phenology

Kathleen Donohue, George Chiang, Deepak Barua, Colleen Colleen, Shane Heschel
Harvard University

Germination is influenced by seasonal environmental factors experienced during seed maturation on the maternal plant. Both the photoperiod and the temperature during seed maturation alter germination responses to post-dispersal conditions. The response of phytochrome mutants to cool maturation temperatures differed from that of the wild-type, indicating a role of phytochrome in maternal temperature effects on germination. NILs containing different natural allelic variants of the flowering-time gene, FLC, also differed in their germination response to maternal temperature. NILs containing different natural variants of a known dormancy gene, DOG1, differed in their germination response to maternal photoperiod. These are among the first genes to be associated with maternal seasonal effects on germination. Results suggest that genes known to be involved in the regulation of flowering time—phytochromes and FLC—are also likely to be involved in regulating germination phenology, and that natural variation exists for germination responses to maternal seasonal factors that vary with flowering time.

357 Association Mapping of Shade Avoidance Responses in Arabidopsis thaliana

Daniele Filiault[1], Carolyn Wessinger[1], Keyan Zhao[2], Magnus Nordborg[2], Julin Maloof[1]

[1]University of California, Davis, [2]University of Southern California

Natural variation in developmental responses to light quality has been shown to have adaptive value. The goal of this work is to assess the genetic basis of variation in phytochrome B mediated responses through association mapping using both candidate gene and whole genome scan approaches. To this end, 95 Arabidopsis thaliana accessions were grown under high and low red:far-red ratios to simulate shade and sun conditions. Plants were phenotyped for shade avoidance and hypocotyl elongation responses. Regression analysis was then used to examine associations between these phenotypic parameters and polymorphisms in loci known to play a part in phytochrome B responses, including eight polymorphisms in phytochrome B itself. Variation in both shade avoidance and hypocotyl elongation phenotypes was observed among the accessions and significant associations between this variation and a number of candidate gene polymorphisms were found. These results suggest that variation in many different loci contributes to the observed phenotypic variation. A genome-wide scan to identify potential novel loci involved in phytochrome B mediated responses was also performed using a marker set spaced at about 50kb intervals throughout the genome.

358 Comparative Genomics of the *Arabidopsis thaliana* and *Oryza sativa BTB* Gene Superfamilies.

Derek Gingerich[1], Shin-Han Shiu[2], Richard Vierstra[1]

[1]University of Wisconsin - Madison, [2]Michigan State University

Selective modification of proteins by ubiquitination is directed by diverse families of ubiquitin protein ligases (or E3s). A large collection of E3s use Cullins (CULs) as scaffolds to form multisubunit E3 complexes in which the CUL binds both a target recognition subcomplex and the RBX1 docking protein which delivers the activated ubiquitin moiety. In one family, CUL3 proteins associate with RBX1 and members of the Broad Complex/Tramtrack/Bric-a-Brac (BTB) protein family to form BTB E3s. Analysis of the *BTB* families in eukaryotes has shown that there is significant diversity with respect to domain architecture between plants, nematodes, arthropods, and vertebrates, and, further, that there have been independent expansions of subfamilies within different lineages. This suggests that evolution of E3 target-recognition proteins may be closely tied to diversification of targets as lineages evolve.

We previously described a family of 80 *BTB* genes in *Arabidopsis* and showed that members of the family are able to interact with *At*CUL3a and *At*CUL3b. Here we describe identification and analysis of the 150-member rice *BTB* superfamily. Comparison of the two superfamilies shows they are very similar in domain architectures and subfamily sizes, implying they regulate functions common to both monocots and eudicots. The primary difference occurs in the MATH-BTB subfamily, where in rice there has been a dramatic expansion of the subfamily (77 members) compared to *Arabidopsis* (6 members). Phylogenetic, gene structure, and expression analysis shows that 4 of the rice *MATH-BTB* loci fall into a core group with the 6 *Arabidopsis* members, suggesting that these genes have functions conserved between the two species. The remaining *MATH-BTB* genes in rice are significantly different, the majority sharing a single-exon gene structure and most arranged in large tandem duplication blocks. In addition to the 150 functional *BTB* loci, we also identified 41 putative *BTB* pseudogenes, 39 of which are in the *MATH-BTB* subfamily. The large expansion and high number of pseudogenes in this subgroup suggests rapid birth and death evolution.

359 eQTL-mapping reveals *AMP2A*, a circadian regulated dioxygenase affecting rhythmic leaf movement

Samuel Hazen[1], Tatjana Singer[2], Yiping Fan[3], Steven Briggs[4], Steve Kay[1]

[1]The Scripps Research Institute, [2]The Salk Institute, [3]St. Jude Children's Research Hospital, [4]University of California, San Diego

Organisms have evolved to coordinate their activities with the day-night cycle caused by the Earth's rotation with an internal mechanism known as the circadian clock. Anticipating environmental changes rather than simply reacting to them increases photosynthesis, growth, and survival. *Arabidopsis* accessions often exhibit diverse circadian properties that are under genetic control. We combined high-density array genotyping and transcription profiling the Col/L*er* recombinant inbred line population to explore this natural variation and identified an eQTL associated with leaf movement rhythms. The expression of a putative dioxygenase located in a previously mapped QTL was strongly correlated with the amplitude of leaf rhythms. We discovered that the Col allele of the putative causal gene is rhythmically expressed while the L*er* allele, which has a positive effect on amplitude, is not expressed at all. The expression polymorphism occurs in at least one third of all accessions tested and is likely caused by a 23bp deletion in the upstream regulatory region. Moreover, a loss-of-function mutant in Col exhibited increased amplitude and thus confirmed the phenotype association. Flowering time and the expression of several core clock genes are not altered in the loss-of-function mutant; therefore, the putative dioxygenase appears to be part of circadian regulated output pathway.

360 Arabidopsis/oomycete symbioses provide a model for phylogenomics and molecular epidemiology in plants

Eric Holub

University of Warwick

Much progress has been made from molecular genetics of disease resistance, with Arabidopsis thaliana pathology at the forefront in revealing parallels with innate immunity of animals. Since coevolution emerged as a research topic more than 25 years ago, there has been general agreement that disease resistance evolves within plant species by a treadmill of balancing selection involving parasite recognition proteins encoded by R-genes and matching "avirulence" effectors (AVR genes) in the parasite (1). Evidence for an adaptive "arms race" driving speciation of the host as well as the parasite has yet to be revealed. This may begin to change with phylogenetic analyses of host genes that govern basic compatibility in plant-parasite symbioses, or that delimit host specialization of parasite taxa. Ellingboe (2) defined basic compatibility as an evolved state that exists between taxa (species or subspecies) of a plant and a parasite that has adapted to survive and proliferate in that host. The adapted parasite must be equipped to evade or overcome constitutive barriers and inducible defenses of its compatible host, and possess the developmental and metabolic characteristics necessary to exploit its nutritious environment. He suggested that the evolution of R-AVR mediated resistance is superimposed onto basic compatibility. The parasite secretome of effector-like genes and any known corresponding host R-genes will provide a focus for future research. Genome-wide comparative biology among species of hosts and parasites would constitute phylogenomics of parasitic symbioses, whereas variability within species would enable microevolutionary or molecular epidemiology. Research of Arabidopsis associations with its adapted oomycete parasites (Hyaloperonospora parasitica and Albugo candida) will provide pull-through knowledge into crops and other wild plant species (1). Exciting progress has recently been made in molecular characterisation of effector proteins from H. parasitica, based on recognition by corresponding Arabidopsis R-genes. A large number of effector-like proteins may be gleaned and classified by bioinformatics from a reference genome of H. parasitica (isolate HpEmoy2) and developed for different experimental uses in comparative biology. Some may prove useful for phylogenomics among species, and others may prove most useful for molecular epidemiology.

1 Holub, EB. Curr. Opin. Plant Biol. 2006 in press.

2 Ellingboe, AH. In Physiological Plant Pathology (Encycl. of Plant Physiol., Vol 4). Heitefuss R, Williams, PH (eds): Springer. 1976:761-778.

361 Evaluation of Arabidopsis FOX line: T2 phenotype and phenotype recapitulation by transformation

Takanari Ichikawa[1], Miki Nakazawa[1], Youichi Kondou[1], Akie Ishikawa[1], Mika Kawashima[1], Haruko Iizumi[1], Motoaki Seki[1], Miki Fujita[2], Syu Muto[3], Kazuo Shinozaki[1], Minami Matsui[1]

[1]RIKEN PSC, [2] CREST, JST,, [3]NEC,soft,Ltd.

We describe here the establishment of a novel gain of function system named as Fox hunting system (Full-length cDNA over-expresser gene-hunting system) for random over-expression of a normalized Arabidopsis full-length cDNA library. Full-length cDNAs from Arabidopsis were sequenced and c.a. 10,000 non-redundant clones were randomly expressed in Arabidopsis plants. We observed 1,487 morphological mutant candidates while growing 15,547 T1 transformants. One of the most frequently observed phenotypes was that of pale green leaves and stems. When 115 such T2 lines were observed 59 of these lines showed the pale green phenotype in dominant or semi-dominant fashion. We will introduce 10 different lines that showed the visible phenotypes upon retransformation of the wild type plant with the rescued full-length cDNAs. Applications of this system on rice functional genomics will be discussed.

362 Population Genetic Variation in Flowering Time Response among Seasonal and Geographic Light Conditions

Yan Li, Peter Roycewicz, Justin Borevitz

Department of Ecology and Evolution, University of Chicago

Large genetic variation for the flowering time has been observed in different Arabidopsis thaliana accessions that is influenced by day length. We have collected 1000s of new lines from various Midwestern sites across different times of year. These lines and others are being genotyped for > 100 common SNPs to survey population and seasonal genetic variation. Unique lines will be available and are being phenotyped for flowering time in simulated seasonal conditions representing Spain and Sweeden. Our growth chambers contain far-red, red, true-white, and blue fluorescent bulbs on electronically dimmable ballasts the recreate daily sunrise, bright midday, and sunset conditions. The intensity, light quality spectrum, and day length, changes throughout the year and is more dramatic in Spain vs Sweeden. Temperature and humidity also cycle throughout the day and year accordingly. We have already used these conditions to map Quantitative trait loci (QTL) responsible for spring flowering time in the accessions of Kas/Col RIL set across 2 replicate blocks. A linkage map from 96 RILs (~12 plants each) was established using 119 markers (64 new SNPs and 55previous ones). One major QTL mapping to the *FRIGIDA* (FRI) locus, was detected on the top of chromosome 4 that had differential effects depending on the geographic condition. Other minor QTL were unique to particular geographic locations. These GxE QTL suggest that subtle changes in light quality, rate of change of day length, and temperature are differentially felt by known and novel alleles controlling flowering time and may be responsible for adaptation to regional environments.

363 Progress Towards the Cloning of sw4.1., the Major Gene for Natural Seed Size Variation in Tomato: From Fine Mapping to BAC Sequence Analysis

Cintia Orsi, Steven Tanksley
Cornell University

Seed size is a life-history trait whose significance is emphasized by its ecological, agronomic and evolutionary importance. In nature, seed size is associated with fitness, playing a major role in seed size-number trade-off. Seed size increase was also one of the consequences of the domestication process for most of the crops, including seed crops. Despite the obvious importance of seed size, the genetic and molecular bases for variation in seed size in nature is still largely unknown. Previous studies in tomato have identified a QTL, sw4.1., which accounts for a large portion of the seed size difference between wild and cultivated tomatoes. Nearly isogenic lines have been created for the fine mapping of this seed size QTL. A subsequent screening of 10,000 F2 individuals from a cross between the NILs has allowed us to narrow down the seed-size controlling region to 30 kb within a single BAC clone. A combination of association genetics and transformation/complementation experiments are being used to pinpoint the specific gene involved and the nature of the genetic changes in this gene (or its promoter) which are causally related to natural variation in seed size.

*Supported by grants from USDA-NRI, NSF and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq)/Brazil.

364 Using small molecules to probe natural variation: characterization of strain-specific xenobiotic metabolism in Arabidopsis

Rachel Puckrin, Sean Cutler
University of Toronto

Plants are exposed to a diverse array of xenobiotics (natural and manmade chemicals) that can affect their growth, development and productivity. Additionally, plants frequently metabolize xenobiotics into new forms that are toxic to humans and / or have adverse environmental effects. In spite of its importance, many aspects of plant xenobiotic metabolism are poorly understood at the biochemical level. We are tackling this problem using a genetic approach that aims to define genes involved in xenobiotic uptake, metabolism and excretion in Arabidopsis which we are defining as XEN loci (for xenobiotic disposition loci). To identify XEN loci we have used HPLC coupled to Diode Array Detection (HPLC-DAD) to survey the uptake, metabolism and excretion of ~400 structurally diverse xenobiotics on the Col , Ler and Cvi ecotypes. The compounds selected for analysis by HPLC included approximately 280 compounds identified in a chemical genetic screen as inhibitors of hypocotyl cell expansion and an additional 120 randomly selected compounds that are not active in hypocotyl cell expansion assays. Greater than 85% percent of the compounds analysed are detectable by HPLC-DAD in extracts prepared from drug treated seedlings; of these, ~35% of show evidence of metabolism and ~18% show evidence of excretion. Comparison of HPLC-DAD profiles across the ecotypes surveyed reveals that multiple compounds show strain-specific differences in metabolism; recombinant inbred lines are currently being used to dissect the genetic basis of these differences. Our results suggest that small molecules are effective probes of metabolic differences between ecotypes and thus can be used to expand the toolkit used for dissecting natural variation in Arabidopsis.

365 Exploring the Evolution of Tandemly Duplicated Receptor Like Kinases in *Arabidopsis*

Adriana Racolta[2, 1], *Frans Tax*[1]

[1]Department of Molecular and Cellular Biology, [2]IGERT Program in Genomics, University of Arizona

Almost one in five Arabidopsis genes is found next to their most close relative. Among receptor-like kinases (RLKs) genes, 33% are organized in tandem duplications or larger clusters. We are interested in understanding why tandem duplicated genes are maintained in the genome. Gene fate after duplication can include inactivation, neo-functionalization and sub-functionalization. We chose to compare DNA sequences from a pair of tandem genes, At2g13790 and At2g13800, which are related to the leucine rich repeat (LLR)-RLK BAK1 which participates in steroid signal transduction. Sequence evolution of these two paralogous genes was analyzed in *Col* ecotype using the Ka/Ks ratio for each protein domain. The low rate of nucleotide substitution in the kinase domain in both coding and non-coding regions indicates that gene conversion is probably maintaining the high identity. Stabilizing selection appears to maintain similarities in the LRR domain of the two genes. For a more complete comparison we also sequenced the kinase domain of the two paralogs in the distantly related ecotype *Cvi-0* and compared them with the *Col* sequences as a measure of their divergence. This leads to the finding that within each ecotype the two genes are more closely related to each other than with the gene pair in the other ecotype. A more recent substitution event, R420L in the highly conserved kinase domain of *Col* At2g13800 was not detected in *Cvi-0* indicative of possible inactivation of this gene in *Col* ecotype. Complete sequencing of both genes in *Cvi-0* will allow for a more complete analysis.

366 Wide Variation in Geminivirus-Mediated VIGS of ChlI in Arabidopsis Ecotypes

Miguel Flores-Vergara[2], *John Tuttle*[2], *Susanne Kjemtrup*[1], *Dominique Robertson*[2]

[1]Paradigm Genetics, RTP, NC, US, [2]North Carolina State University, Raleigh, NC US

The geminivirus cabbage leaf curl virus (CaLCuV) has been modified to serve as a transient gene silencing vector for Arabidopsis. In a screening of over 100 different Arabidopsis accessions, extensive variation in the degree of silencing of an endogenous gene (ChlI), needed for chlorophyll formation, was seen. A total of 20 plants from each ecotype was bombarded with the VIGS vector, and the experiment was repeated once. Wile some ecotypes lacked detectable silencing, others showed extensive ChlI silencing. Viral symptoms also varied from minimal to severe, and the two traits were not correlated. The responses were grouped into four general classes, with the most abundant class showing symptoms (stunted growth and leaf curling) and ChlI silencing, and the next most abundant class showing symptoms but no visible ChlI silencing. Several ecotypes showed extensive silencing with minimal symptoms and could be suitable as a host for virus-induced gene silencing. One class lacked both symptoms and silencing and is a possible candidate for a geminivirus resistance gene. In addition to the four general classes of response, two ecotypes were identified that consistently lost ChlI silencing in new growth. Although the screen was performed at 22/20^{o}, short day conditions, four ecotypes with mild symptoms and extensive ChlI silencing were also compared for VIGS response at different temperatures. ChlI silencing was generally greater at 25/23^{o}, but Kil-0 retained extensive ChlI silencing at 16/14^{o} while Le-0 did not and there was minimal effect on symptoms for the four ecotypes. Because the wide variation in response to CaLCuV:ChlI infection occurred in ecologically fit accessions, this system provides a unique opportunity to identify genetic components that mediate epigenetic responses to a DNA virus, and to VIGS of an endogenous gene mediated by that virus.

1. S. Kjemtrup's current address: Monsanto-RTP, Research Triangle Park, NC US

2. Supported by NSF STTR 021503 to SK and DR

367 Natural Variation in Inflorescence Replacement in Arabidopsis

Cecile Sano[2], Martin Bohn[3], Ken Paige[1], Thomas Jacobs[2]

[1]Department of Animal Biology, University of Illinois at Urbana-Champaign, [2]Department of Plant Biology, University of Illinois at Urbana-Champaign, [3]Department of Crop Sciences, University of Illinois at Urbana-Champaign

Animals ultimately obtain all their energy from plants. Herbivory therefore imposes an inevitable selection pressure on every plant species' evolution. Reserving axillary meristems can reduce the fitness cost of losing reproductive structures to browsing herbivores. When an Arabidopsis plant's primary inflorescence is removed or damaged, axillary buds can replace it by elongating into axillary inflorescences. This phenomenon has long been known as apical dominance. We have adopted a quantitative genetic approach in an effort to understand the genetic basis of the Arabidopsis Inflorescence Replacement Program (IRP) and its natural phenotypic variation. We have begun by scoring the responses to apex removal of a geographically diverse collection of 90 Arabidopsis accessions. The primary inflorescence of each plant was clipped at its base when its first flower opened or when it reached a height of 4 cm, whichever came first. Parameters scored included: days to flower (DFL), length of primary inflorescence (APL) at clipping, days to re-flower after clipping (DRF), number of rosette leaves at the time of clipping (LFN), number of axillary inflorescences 0.3 cm or longer on the day of clipping (ACL), and number of axillary inflorescences (ARF) and the length of each (AXL) at the day of re-flowering. The day that the first axillary flower was fully opened was designated the day of re-flowering.

A randomized incomplete block design was used. Plot means based on four plants per plot and accession were calculated and ANOVA, cluster analysis and principal component analysis (PCA) were performed. High heritability was seen for all measured parameters. The parameter pairs DFL/DRF, DFL/LFN and DRF/LFN were positively correlated. AXL/DFL and ACL/DRF were negatively correlated. Five major phenotypic clusters were identified within the 90 accessions. Within-cluster PCA suggests that several distinct IRP strategies are discernable among the 90 accessions, with each IRP strategy composed of a uniquely weighted combination of measured traits. Our hypothesis of differing IRP strategies across Arabidopsis germplasm is therefore supported by this analysis. From these 90 accessions, we have selected for intercrossing a subset that represents varying and extreme IRP strategies. QTL will be mapped for IRP component traits in segregating populations. This analysis will provide insights into the underlying genetic basis, and its global variation, of an adaptive, and possibly co-evolving suite of traits in Arabidopsis. QTL identified may also represent novel loci heretofore unidentified by conventional mutant analyses of branching and apical dominance.

368 The Arabidopsis Biological Resource Center – Current Acquisitions and Activities

Randy Scholl, Emma Knee, Luz Rivero, Deborah Crist, Natalie Case, Heather Joesting, Daniel Johnson, Kate Ludwig, James Mann, Cori Phillips, Garret Posey, Pamela Vivian, Zhen Zhang, LIng Zhou

Arabidopsis Biological Resource Center, Dept. of Plant Cellular and Molecular Biology and Plant Biotechnology Center, The Ohio State University

The Arabidopsis Biological Resource Center (ABRC) collects, preserves and distributes seed and DNA stocks of Arabidopsis. ABRC stock information is in the TAIR (maintained by the Carnegie Institution of Washington, with informatics support from the National Center for Genomic Resources) and stocks can be ordered at (http://arabidopsis.org).

Seed stocks have been added to our collections in the past year, including: A) 1,900 confirmed, purified T-DNA lines from J. Ecker; B) purified T-DNA insertion lines from researchers C) ca. 9,000 SAIL T-DNA from Syngenta, D) T-DNA lines from GABI-Kat; E) RNAi lines from AGRIKOLA, F) mutant lines; G) a recombinant inbred population form J. Borevitz; and H) miscellaneous transgenic lines. The T-DNA lines of the SALK, SAIL and Wisconsin collections provide insertions in 25,000+ different Arabidopsis genes.

New DNA stocks added to the collection include: A) sequence-validated open Reading Frame (ORF) clones from J. Ecker, B) ORF clones from C. Town; C) 1,100 Gateway ™ Expression clones from S. P. Dinesh Kumar, D) multifunctional vectors that utilize the Gateway™ system and E) Expression clones from different researchers. The present ORF and cDNA collection represents 15,000+ genes. The SSP and Salk ORF collections have been formatted to plates and revalidated by end sequencing.

During the past year, ABRC distributed 70,000 seed and 26,600 DNA stocks to researchers. Distribution of T-DNA lines contribute to the very high number of seeds being sent, and the ORF clones represent the most popular DNA stocks.

ABRC is supported by the National Science Foundation.

369 The effects of multiple floral promotion pathways on flowering time in winter-annual Arabidopsis thaliana accessions

Christopher Schwartz, Richard Amasino
UW-Madison

The timing of flowering is key for reproductive success, and plants have elaborate systems to control when a given plant will flower in a specific environment. To better understand how flowering is induced in the wild, we are utilizing the natural variation in flowering time of Arabidopsis accessions collected from around the world. We have surveyed over 300 natural accessions of Arabidopsis for flowering time and determined that approximately 45% of Arabidopsis accessions are late flowering (winter-annuals). A subset of accessions was chosen based upon phenotype and geographic location to represent the flowering time variability observed in nature. We tested the effects of three different floral promotion pathways on these accessions to gain an understanding of the role each pathway plays in promoting flowering in different accessions, which are presumably adapted to different local environments. To determine the effect of the shade avoidance response, plants were grown under cool white light supplemented with far-red light. For characterizing the vernalization response, seedlings were cold treated for variable lengths of time and the effect on subsequent flowering time determined. Finally, the plant hormone GA was applied to the accessions to assay the responsiveness of these accessions to GA induced flowering. We found substantial variability in the effects of a given treatment on flowering time; in addition the effects across the multiple treatments were poorly correlated, supporting the idea that these are largely independent or separate pathways. Based upon this data, we generated multiple F2 populations from accessions that display contrasting behavior for sensitivity to a given floral promotion pathway. In some cases, we see Mendellian segregation, implying that a single locus is responsible, while in other F2 populations, we see a continuous phenotypic distribution suggesting multiple causative loci. In the far-red supplementation experiments, designed to elicit the shade avoidance response, all the F2 populations segregated in a 3:1 manner, suggesting that a single large effect locus was responsible for the differences in sensitivity to far-red light. Preliminary mapping using multiple segregating populations indicates that the same locus is responsible for the far-red sensitivity in many different accessions. In addition, when the same accessions are grown outside in the summer in Wisconsin, the outdoor flowering phenotype is highly correlated to the flowering time observed when far-red light supplementation is included, suggesting that this response is physiologically relevant and important for reproductive success in the wild.

370 High resolution mapping of genome variations between polyploid and diploid *Arabidopsis* species

Shin-Han Shiu[1], Xu Zhang[2], Justin Borevitz[2]
[1]Michigan State University, [2]University of Chicago

Polyploidization results in an immediate increase in gene dosage and essentially releases the whole genome from purifying selection. Based on studies of paleopolyploidy in plants and yeasts, most genes are lost within tens of millions of years of the polyploidization event but some seem to be preferentially retained. We are interested in understanding the differential retention of genes and the rate of gene loss in polyploid species. However, without polyploid genome sequences, the extent and the nature of sequence variation in these species are virtually unknown. We set out to obtain a high resolution map of sequence variation in a relatively recently formed polyploid, *Arabidopsis suecica* (*As*) by comparing *As* to its parental species, *Arabidopsis thaliana* and *Arabidopsis arenosa*. The genomic DNA of *As* and its parents was isolated and hybridized independently to *Arabidopsis* whole genome tiling arrays, 4 biological replicates each. Among > 3 million features on the tiling array, 112,035 are significantly different between *As* and its parental species. Although we predicted that hybridization intensities should be higher for the parental genomes due to gene losses in the polyploid, nearly half of the variable features have intensities significantly higher in *As*. Most (60%) of the sequence variation is present in the intergenic regions. As expected, genic features tend to be less variable than intergenic ones. However, pseuogenes, retroposons, and transposons have significantly higher proportions of variable features (after normalizing by number of features) than intergenic sequences, suggesting they may have been purged or deleted soon after the polyploidization event. Among features located within genic regions, those overlapping with tandemly duplicated genes also tend to have a higher level of variation. In addition, many genes with significantly more variable features are related to those preferentially lost since the most recent paleo-polyploidization event in *Arabidopsis* (~25-45 Mya). Assuming these highly variable genes have been deleted in *As*, a polyploid formed ~20,000 years ago, our finding suggests that differential gene loss occurs rather rapidly after the polyploidization event.

371 Natural Variation for Seed Dormancy in Arabidopsis thaliana

Chunlao Tang, Keyan Zhao, Magnus Nordborg
University of Southern California

Seed dormancy is an important adaptive trait, primarily due to its effect on seedling survival. However, little is known about natural variation for this trait, both because of complex genetics and because of difficulties in obtaining reliable phenotypic measurements. Here we report the results of a survey of primary seed dormancy in a world-wide collection of 96 Arabidopsis thaliana accessions, for which genome-wide polymorphism data are also available.

We found extensive variation of seed dormancy among accessions and demonstrated that the requirement for after-ripening for seed germination (specifically, the number of days of dry storage required to reach 50% germination ratio) is a feasible index of this trait in a large-scale survey as long as experimental conditions are strictly controlled. Overall, no significant association was found between seed dormancy and latitude, temperature or flowering time. However, the geographic distribution of seed dormancy was evidently non-random. The strongest seed dormancy was found in accessions from regions with dry summers and/or harsh winters such as the Mediterranean or Central Asia. At the other extreme, all seeds were found to be non-dormant in accessions from northern Sweden, where prompt germination might be advantageous due to the very short growing season. In general, large variation in seed dormancy was found between accessions from very close origins, suggesting considerable micro-geographic heterogeneity.

372 Sucrose Specific Induction of Anthocyanin Biosynthesis in Arabidopsis Requires the MYB75/PAP1 Gene

Sheng Teng[1], Joost Keurentjes[2], Leonie Bentsink[1], Maarten Koornneef[2], Sjef Smeekens[1]
[1]Department of Molecular Plant Physiology, Utrecht University, the Netherlands, [2]Laboratory of Genetics, Wageningen University, the Netherlands

Sugar-induced anthocyanin accumulation has been observed in many plant species. We observed that sucrose is the most effective inducer of anthocyanin biosynthesis in Arabidopsis seedlings. Other sugars and osmotic controls are either less effective or ineffective. Analysis of sucrose induced anthocyanin accumulation in 43 Arabidopsis accessions shows that considerable natural variation exists for this trait. The Cvi accession essentially does not respond to sucrose, whereas Ler is an intermediate responder. The existing Ler/Cvi recombinant inbred population was used in a QTL analysis for sucrose induced anthocyanin accumulation. A total of four quantitative trait loci for sucrose induced anthocyanin accumulation (SIAA) were identified in this way. The locus with the largest contribution to the trait, SIAA1, was fine mapped and using a candidate gene approach, it was shown that the MYB75/PAP1 gene encodes SIAA1. Genetic complementation studies and analysis of a laboratory-generated knockout mutation in this gene confirmed this conclusion. Sucrose, in a concentration dependent way, induces MYB75/PAP1 mRNA accumulation. Moreover, MYB75/PAP1 is essential for the sucrose-mediated expression of the DFR gene.

The SIAA1 locus in Cvi probably is a weak or loss of function MYB75/PAP1 allele. The C24 accession similarly shows a very weak response to sucrose induced anthocyanin accumulation encoded by the same locus. Sequence analysis showed that the Cvi and C24 accessions harbor mutations both inside and downstream of the DNA binding domain of the MYB75/PAP1 protein, which most likely result in loss of activity.

373 An Inventory of Common Sequence Polymorphisms for *Arabidopsis*

Richard Clark[1], Stephan Ossowski[1], Gabriele Schweikert[1, 2], Georg Zeller[3], Paul Shinn[4], Gunnar Raetsch[5], Norman Warthmann[1], Glenn Fu[6], David Hinds[6], Huaming Cheng[4], Kelly Frazer[6], Chris Toomajian[7], Tina Hu[7], Daniel Huson[3], Bernhard Schoelkopf[2], Magnus Nordborg[7], Joseph Ecker[4], Detlef Weigel[1]

[1]Department of Molecular Biology, Max Planck Institute for Developmental Biology, Spemannstrasse 37-39, Tuebingen, Germany, [2]Max Planck Institute for Biological Cybernetics, Spemannstrasse 38, Tuebingen, Germany, [3]Center for Bioinformatics Tuebingen, Tuebingen University, Tuebingen, Germany , [4]Genomic Analysis Laboratory, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA, USA, [5]Friedrich Miescher Laboratory of the Max Planck Society, Max Planck, Spemannstrasse 35, Tuebingen, Germany, [6]Perlegen Sciences, 2021 Stierlin Court, Mountain View, CA, USA, [7]Molecular and Computational Biology, University of Southern California, Los Angeles, CA, USA

We have used high-density oligonucleotide arrays to identify common sequence variation in 20 wild strains of ***Arabidopsis thaliana*** that were chosen for maximal genetic diversity. The entire 119 Mb reference genome was tiled on the arrays, which were hybridized with whole genome, isothermally amplified DNA to minimize ascertainment biases. Using two approaches, a model based algorithm, and a newly developed machine learning method using Support Vector Machines, we identified over 550,000 single nucleotide polymorphisms (SNPs) with a false discovery rate of ~ 0.03 (an average of 1 SNP for every 216 bp in the genome). A heuristic algorithm also predicted ~700 highly polymorphic or deleted regions in each accession. Nearly 700 SNP or deletion polymorphisms predicted to cause major functional effects (e.g., premature stop codons, or deletion of coding sequence) were validated by traditional methods. Using this data set, we provide the first systematic description of the types of genes that harbor major effect polymorphisms in natural populations at moderate allele frequencies. The data also provide an unprecedented resource for the study of genetic variation in an experimentally tractable, multicellular reference organism.

374 The GABI-Kat Arabidopsis thaliana T-DNA Mutagenised Population for Flanking Sequence Tag-Based Reverse Genetics: 2006 Update

Mario Rosso, Yong Li, Prisca Viehoever, Bernd Weisshaar

Bielefeld University

The GABI-Kat population of T-DNA mutagenised Arabidopsis thaliana lines with sequence-characterised insertion sites is used extensively for efficient progress in plant functional genomics. T-DNA insertion mutants were created in the accession Columbia (Col-0). DNA from leaves of T1 plants was extracted and used for the production of flanking sequence tag (FST) data. About 107,500 FSTs were submitted to EMBL/GenBank/DDBJ and are also available from the GABI-Kat website (http://www.gabi-kat.de). This website also gives access to information about which mutant allele was present in which line, and about the predicted location of the insertion site relative to the given gene. Recent studies indicate that insertion sites appeared significantly more frequent in regions before transcription start and after poly(A)-addition sites of predicted genes. Also, a "restriction site bias" was detected that explains for a fraction of the untagged genes why they are not covered with FST-indexed mutants (Li, Rosso, Ulker and Weisshaar, Genomics 2006, in press).

In addition to serving the community with confirmed insertion mutants, we have started to transfer the most relevant GABI-Kat lines to the Nottingham Arabidopsis Stock Centre (NASC; http://www.arabidopsis.info/). This was made possible by an agreement between the funding partners of GABI and the Max-Planck-Society (MPG) who announced in June 2005 that GABI-Kat lines will become freely available to the international research community. As of March 2006, 2,282 sets of T3 seed that represent one confirmed line each have been delivered to NASC. These about 2,300 sets consist of more that 34,500 individual seed stocks which include in most cases a homozygous stock for a given insertion allele. The number of GABI-Kat lines available from NASC will continue to grow.

375 Natural genetic and epigenetic effects on gene expression and splicing variation in Arabidopsis thaliana using whole genome tiling arrays

Xu Zhang[1], Shinhan Shiu[2], Justin Borevitz[1]

[1]Ecology and Evolution, University of Chicago, Chicago, IL 60634, [2]Plant biology, Michigan State University, East Lansing, MI 48824

Natural variation in gene expression has a heritable component and has been explored in model organisms in inbred lines and controllable environments. Gene expression variation is composed of steady state transcript level differences, alternative spliced transcripts and allele specific expression differences in F1 hybrids. This is the result of cis and/or trans regulatory polymorphisms caused by underlying genetic and epigenetic variation. We have investigated the causes and effects of this variation using whole genome oligonucleotide tiling arrays. Over 100,000 Single Feature Polymorphisms (SFPs) between two wild Arabidopsis accessions, Van and Col , were revealed by genomic DNA hybridization. Subsequently true natural variation in gene expression and alternative splicing was analyzed accounting for SFP variation. A linear model was fit testing main effects of overall transcriptional differences and differences among transcript fragments (exons) for each gene, revealing ~ 2,300 gene expression polymorphism and ~2,100 exon level polymorphisms (FDR 5%). Comparison of the transcript abundance among parental lines and F1 hybrids allows the detection of additive, dominance, and maternal gene effects to be jointly estimated. cis-regulatory polymorphisms and imprinting effects were also analyzed at the genome wide level by testing for allele specific expression in reciprocal F1 hybrids. SFPs within a transcribed fragment are used to distinguish which allele is expressed (Ronald et al 2005, Borevitz et al 2005). Upstream SFPs linked to expression variation are candidates for causative cis regulatory elements in intergenic regions. When variation in expression is not determined by genotype but rather the parental or maternal direction of the cross this allelic expression suggests imprinting effects. Genome wide cytosine methylation patterns were also profiled in parental and hybrid lines to suggesting abundant underlying epigenetic variation which may also contribute to such allelic expressional difference. Current results and methods will be presented (see also http://natural.uchicago.edu/~xuzhang/expression.html).

376 Strain Selective Drugs: Exploiting Natural Variation in Chemical Genomics

Yang Zhao, Freeman Chow, Rachel Puckrin, Karen Ng, Simon Alfred, Pauline Fung, Sean Cutler

Department of Botany, University of Toronto

Natural genetic variation within a species can be documented at many levels of analysis ranging from simple "aphenotypic" molecular polymorphisms to large scale differences in development such as flowering time. One facet of natural variation explored primarily in humans is pharmacogenetic variation, a clinically important from of inter-individual variation in drug sensitivity. We reasoned that if pharmacogenetic variation is biologically pervasive it could be used to identify genetic factors that modulate drug sensitivity in model systems. To survey pharmacogenetic variation in Arabidopsis, several geographically diverse ecotypes were subjected to the same chemical genetic screen of ~13,500 small molecules library to identify compounds that show differential effects on hypocotyl cell elongation. This screen uncovered 5 loci that act as simple Mendelian traits to modify sensitivity to 5 structurally unrelated cell expansion inhibitors.

To gain insight into the molecular mechanisms of pharmacogenetic variation in plants, we have begun mapping and cloning the pharmacogenetic loci identified. We will describe our work on the HYR1 locus, which confers resistance to hypostatin, a new small molecule inhibitor of cell expansion that ~25% of Arabidopsis isolates are naturally resistant to. Genetic analysis, map based cloning and biochemical analyses have shown that most hypostatin resistant strains carry recessive mutations in HYR1, a glycosyl-transferase that converts hypostatin from a pro-drug into an activated form by glycosylation. Intriguingly, HYR1 is part of the large UGT-superfamily of enzymes that play important roles as pharmacogenetic factors in humans. Thus, intraspecific variation in UGT function acts to modulate drug sensitivity across biological kingdoms suggesting that Arabidopsis may be a good model for exploring the mechanisms of pharmacogenetic variation. Additionally, our results demonstrate that small molecules are effective tools for exposing and characterizing natural variation.

377 *Myo*-Inositol Oxygenase Is a Balance Point Between Signaling and Metabolism

Shannon Alford

Virginia Polytechnic Institute and State University

Myo-inositol is used in plant cells as a backbone of inositol phosphate (InsP) and phosphatidylinositol phosphate (PtdInsP) signaling molecules. Myo-inositol is also a precursor for an alternative Vitamin C synthesis pathway in plants. The oxidation of myo-inositol by the enzyme Myo-Inositol OXygenase (MIOX) produces D-glucuronic acid, which is further altered to produce Vitamin C. The Arabidopsis genome contains four genes predicted to encode MIOX enzymes. Recently it was shown that plants ectopically expressing the MIOX4 gene from Arabidopsis ($MIOX4^+$ plants) produced higher levels of Vitamin C. Since MIOX oxidizes myo-inositol, it could act as a central regulator in balancing the cell's various needs for myo-inositol. Specifically, we are interested in whether $MIOX4^+$ plants contain alterations in inositol signaling. We found that $MIOX4^+$ seedlings are hyposensitive to abscisic acid (ABA) in a seedling germination assay. This suggests that $MIOX4^+$ plants are altered in the ABA response pathway. Since ABA signaling has been shown to induce Ins(1,4,5)P_3 synthesis, this response may indicate that $MIOX4^+$ plants are compromised in their ability to synthesize Ins(1,4,5)P_3. T-DNA insertional knock-outs of the four MIOX genes have been obtained. We are examining these mutants for phenotypes and signaling alterations. Specifically, we are measuring myo-inositol levels and other metabolites by GC analysis in $MIOX4^+$ and miox- mutant plants to determine if levels are altered. Ins(1,4,5)P_3 levels will be measured to determine if this signaling molecule is altered. Our results will help us understand how MIOX function impacts myo-inositol signaling and metabolism.

378 The role of the SLEEPY1 (SLY1) F-box gene in GA regulation of seed germination in Arabidopsis

Tohru Ariizumi[1], Elizabeth Schramm[1], Camille Steber[1, 2]

[1]Washington State University, [2]USDA-ARS, Wheat Genetics Unit

Seed germination is a complex developmental process regulated by phytohormones. The phytohormone abscisic acid (ABA) inhibits seed germination, whereas gibberellin (GA) stimulates seed germination. In tomato and Arabidopsis, GA is clearly required for seed germination. Recent evidence suggests that GA stimulates seed germination by triggering destruction of DELLA family proteins via the SCF^{SLY1} E3 ubiquitin ligase and the 26S proteosome pathway. DELLA proteins are negative regulators of GA responses, and RGL2 is the main DELLA protein repressing seed germination. *SLY1* appears to tranduce the GA signal by triggering DELLA destruction by ubiquitination. GA-insensitive sly1 mutants resemble GA biosynthesis mutants in that they exhibit dwarfism, late flowering, reduced fertility and increased seed dormancy. These *sly1* phenotypes are not rescued by GA application and are not as severe as those seen in the *ga1-3* GA biosynthesis mutant. While the *ga1-3* mutant fails to germinate in the absence of GA, the seed germination rate varies greatly (3-100%) among independent seed lots of young sly1 mutants. When sly1 mutant seeds can germination, they germinate more slowly than WT and show greater sensitivity to ABA and reduced osmotic potential. The germination of dormant *sly1* mutant seed lots improved following afterripening. Consistent with the notion that *SLY1* regulates seed germination, a *SLY1* promoter::GUS fusion shows expression in the radicle during seed germination. To better understand the *sly1* mutant seed germination phenotype, we are examining the effect of these mutations on RGL2 protein accumulation. It is known that high levels of RGL2 protein in the *ga1-3* mutant correlates with failure to germinate, and that mutations in *RGL2* suppress the *ga1-3* seed germination phenotype.

379 SPIKE1 is a guanine nucleotide exchange factor that positively regulates ROP small GTPases and controls an evolutionarily conserved pathway of actin-dependent cell morphogenesis

Dipanwita Basu, Taisiya Zakharova, Eileen Mallery, Daniel Szymanski
Purdue University

During cell morphogenesis the actin cytoskeleton is dynamically reorganized in response to endogenous signals. The recently identified WAVE-ACTIN RELATED PROTEIN (ARP) 2/3 pathway clearly illustrates this point. Activating signals from the small GTPase ROP are thought to activate the heteromeric WAVE complex, which in turn, positively regulates the actin filament nucleation activity of ARP2/3. However, several aspects of this signaling pathway are not defined. For example, which of the 11 Arabidopsis ROPs feed into the WAVE-ARP2/3 pathway and which guanine nucleotide exchange factor(s) (GEF) promote GTP-loading of ROP upstream of the WAVE-ARP2/3? We have used a forward genetic analysis of epidermal morphogenesis to define completely, a pathway of genes from GEF to ARP2/3-dependent actin filament nucleation. These genes play important roles in polarized growth and cell-cell adhesion in a variety of tissues and organs. SPIKE1 (SPK1) is the lone plant gene product that contains a DHR2 domain, the defining feature of the DOCK family of GEFs. We will present strong genetic and biochemical data indicating that the DHR2 domain of SPK1 is critical, and that DHR2 is necessary and sufficient for activation of ROP small GTPases in vivo. Using double mutants analyses and the well known wave and arp2/3 phenotype of trichome swelling, we find that one function of SPK1 is to positively regulate this evolutionarily conserved actin filament nucleation pathway; a pathway that appears to utilize only a subset of ROPs. In Rho-signaling pathways, it is extremely difficult to link directly GEF activity to specific Rho-GTP effector targets. For example, multiple ROPs and GEFs could lie between SPK1 and the ROP-binding WAVE protein SRA1. We tested SPK1 function as a "signaling scaffold" for downstream effectors by assaying the ability of endogenous SPK1 to interact with WAVE complex proteins. We will present several lines of biochemical evidence that support the hypothesis that endogenous SPK1 is physically associated with WAVE complex proteins. The SPK1-WAVE complexes may be poised to receive and transmit ROP signals with a high degree of spatial and temporal specificity. To our knowledge this is the first example in which the in vivo function of a signaling pathway from upstream GEF to actin filament nucleation machinery has been defined in a multicellular organism.

380 The Arabidopsis Aleurone Layer Contributes to Seed Dormancy and Responds to GA, ABA and Nitric Oxide

Paul Bethke[1, 2], Natsuyo Aoyama[3], Igor Libourel[4], David Still[3], Russell Jones[2]
[1]USDA ARS Dept of Horticulture, University of Wisconsin, Madison, USA, [2]Dept. Plant and Microbial Biology, University of California, Berkeley, USA, [3]Dept Plant Sciences, California State Polytechnic University, Pomona, USA, [4]Michigan State University, East Lansing, USA

Seed dormancy is a complex phenomenon influenced by both genetic and environmental factors. ABA is central to the establishment and maintenance of seed dormancy, and gibberellins are important for germination. The volatile signaling molecule nitric oxide (NO) reduces seed dormancy in Arabidopsis. Seeds of Arabidopsis are dormant at maturity, and depending on the ecotype and environment, dormancy loss occurs within a few weeks or after several months. Arabidopsis seeds contain an embryo enveloped in an aleurone layer that is in turn surrounded by the testa. Imbibed Arabidopsis seeds remained dormant when the testa was removed, but like intact seeds germinated when treated with KCN vapors or NO gas. Removal of both the testa and the aleurone layer resulted in growth of the embryo. Aleurone cells underwent characteristic changes in ultrastructure in seeds that will germinate, and the most obvious was a reduction in the number of vacuoles per cell. The rate of this vacuolation was greatest for the cells proximal to the root tip. Vacuolation did not occur in dormant seeds and was prevented by ABA or the NO scavenger cPTIO. The effect of cPTIO was overcome by GA, and GA on its own promoted the vacuolation of aleurone cells. These data suggest that the aleurone layer is a significant determinant of Arabidopsis seed dormancy, and that this tissue is responsive to ABA, GA and NO in ways that are consistent with the physiology of dormancy and germination in this species.

We also quantified mRNA abundance of key genes associated with dormancy and germination in the aleurone layer and embryo of Arabidopsis seeds. Dormant seeds of ecotype C24 were imbibed with water or with the nitric oxide (NO) scavenger cPTIO, conditions that maintain or strengthen dormancy respectively. Other seeds were treated with KCN vapors to break dormancy in an NO-dependent manner. Embryos and aleurone layers were dissected from seeds and quantitative PCR was used to measure mRNA abundance at 1, 24 and 48 h after imbibition for genes associated with GA biosynthesis (GA3ox1, GA30x2), GA responses (cysteine protease), lipid metabolism (malate synthase, isocitrate lyase), and NO synthesis (nitric oxide synthase). Our data show that genes for GA biosynthesis were strongly up-regulated in the embryos of seeds that will germinate relative to seeds that will remain dormant. GA3ox1 and GA3ox2, however, were not expressed at detectable levels in the aleurone layer, even at times when a GA-responsive cysteine protease was expressed. The nitric oxide synthase was expressed in both the embryo and the aleurone layer, and expression in the aleurone layers was strongly stimulated by the NO scavenger cPTIO.

381 The Auxin Perception Network

May Christian[1], Hartwig Luethen[2]

[1]University of North Carolina at Chapel Hill, [2]University of Hamburg

During the last years the number of genes identified to be involved in auxin responses raised constantly as did the number of related mutants. In contrast characterization of these mutants in regard of the most relevant part of auxin action – the growth response – is still incomplete at best. The reason is that *Arabidopsis* organs displaying auxin-induced growth are so tiny and that auxin responses occur on short time scales. To deal with these problems we developed a CCD-based method in order to carry out high-resolution growth measurements of *Arabidopsis hypocotyls* and flower stems.

We used this novel technique to investigate the very first part of auxin signaling - auxin perception - and revealed apparently paradox results.

Back in the 1990ies there was some physiological evidence pointing to an intracellular auxin receptor. Analysis of the auxin influx carrier mutant *aux1* confirmed the belief that auxin perception takes place inside the cell. We were able to support this theory by observing the growth response of *tir1* with the help of the CCD-auxanometer.

On the other hand auxin perception was generally regarded as a process localized at the cell surface. A putative auxin receptor, auxin binding protein 1 (ABP1), has been identified. Unfortunately knocking out this gene results in embryo lethality. To by-pass this problem we developed another strategy using signaling mutants to explore if auxin signaling chains starting with ABP1 are involved in growth control. Here we used a single cell system responsible for auxin and analyzed the physiological effects of anti-ABP1 antibodies. We found that there is at least some linkage between ABP1 and growth control.

We will propose hypotheses to bring these at first view puzzling results together.

382 MUBS: a Family of Ubiquitin-Fold Proteins that are Plasma Membrane-Anchored by Prenylation

Brian Downes[1], Scott Saracco[2], Sang Sook Lee[2], Dring Crowell[3], Richard Vierstra[2]

[1]Saint Louis University, [2]University of Wisconsin-Madison, [3]Indiana U. Purdue U. Indianapolis

Ubiquitin (Ub)-fold proteins are rapidly emerging as an important class of eukaryotic modifiers, which often exert their influence by post-translational addition to other intracellular proteins. Despite assuming a common β-grasp three-dimensional structure, their functions are highly diverse owing to distinct surface features and targets, and include tagging proteins for selective breakdown, nuclear import, autophagic recycling, vesicular trafficking, polarized morphogenesis, and the stress response. Here, we describe a novel family of Membrane-anchored Ub-fold (MUB) proteins that are present as single copy genes in animals and filamentous fungi and small gene families in plants. Extending from the C-terminus of the Ub fold is typically a cysteine-containing CaaX box, a canonical signal for the attachment of either a 15-carbon farnesyl or a 20-carbon geranylgeranyl moiety.

Using *in vitro* prenylation assays, we show that representative MUBs from humans, mice, *Drosophila, Xenopus,* zebrafish, and *Arabidopsis* can be prenylated, and the cys of the CaaX box is required for prenylation. Modified forms of several MUBs were detected in transgenic *Arabidopsis*, suggesting that these MUBs are prenylated *in vivo*. Both cell fractionation and confocal microscopic analyses of *Arabidopsis* plants expressing GFP-MUB fusions showed that the modified forms are membrane anchored with a significant enrichment on the plasma membrane. This localization could be blocked by mevinolin, which inhibits the synthesis of prenyl groups. In addition to the five MUBs with CaaX boxes, *Arabidopsis* has one unique MUB variant with a cysteine-rich C-terminus distinct from the CaaX box that is also membrane anchored, possibly through the attachment of a long-chain acyl group. While the physiological role(s) of MUBs remain unknown, the discovery of these prenylated forms further expands the diversity and potential functions of Ub-fold proteins in eukaryotic biology.

383 The RAD23 Family of Ubiquitin-Like Proteins Regulates Plant Development and Abscisic Acid Signalling in *Arabidopsis thaliana*

Lisa Farmer[1], Hong-Yong Fu[2], Richard Vierstra[1]

[1]Department of Genetics, University of Wisconsin-Madison, Madison, WI 53706, USA, [2]Institute of Plant and Microbial Biology, Academia Sinica, Nankang, Taipei, Taiwan 11529

The Ubiquitin/26S Proteasome System (UPS) is the preferential mechanism by which transient regulatory or misfolded proteins are discarded. Ubiquitin (Ub) is covalently attached to target proteins by a series of E1-E2-E3 enzymatic activities, and conjugates are delivered to the 26S proteasome, where they are unfolded and degraded. Ub conjugation and proteolysis have been described, but less is known about the identities of specific targets and how they are transported to the proteasome. Evidence suggests that RAD23 proteins are responsible for delivering targets to the proteasome. RAD23s have N-terminal Ub-like (UBL) and C-terminal Ub-associated (UBA) domains that facilitate their interactions with the proteasome and poly-Ub chains, respectively. We predict that these associations allow RAD23s to accept ubiquitin conjugates and then "dock" with the proteasomal subunit RPN10 to relinquish their cargo. The Arabidopsis RAD23 family includes four highly conserved members. By analysis of genetic knockouts in the four AtRAD23 genes, we found that they are important for appropriate root, shoot and reproductive organ development in seedlings and mature plants, respectively. The AtRAD23s preferentially bind Lys48-linked poly-ubiquitin chains, which are a signature for degradation. They also interact with RPN10, which helps regulate the levels of hormone signaling factors such as the ABA transcription factor ABI5. We have evidence that ABI5 levels are affected by multiple AtRAD23s. Further analysis should reveal other functions of the AtRAD23s, as well as the identities of important developmental regulators that are recycled by the UPS.

384 Characterization of Gal4-mediated driver lines

Karen Fitzsimmons, Melissa Curran, Robert Kranz

Washington University in St. Louis

Arabidopsis lines have been generated that contain three reporter genes (LUC, GFP, GUS) that are under the control of Gal4, since the 5X UAS that binds Gal4 was cloned upstream of each reporter. A variety of Gal4 driver lines have been identified based on tissue location of reporter expression. To express a gene ectopically, driver lines can be crossed with responder lines, plants that have a gene of interest with 5X UAS. We have used the CAPRICE or CPC transcription factor to test the driver/responder system. The CPC over expression phenotype (with a full 35S promoter) includes increased root hairs and reduced trichomes. These multiple tissue-specific phenotypes make CPC an ideal transcription factor to test. Several different Arabidopsis driver lines have been transformed with 5X UAS:CPC and phenotypes correlated with expression. A quantitative analysis that correlates expression of the three reporters to phenotypic expression will be presented.

385 Identification and Characterization of a Novel, Recessive Allele of CUL1 that Disrupts SCF regulation at the C-terminus of CUL1

Jonathan Gilkerson[1, 2], Jessica Brown[2], Judy Callis[2, 1]

[1]UC Davis, Plant Biology Graduate Group, [2]UC Davis, Section of Molecular and Cellular Biology

Auxin regulates many aspects of plant growth and development and has been shown to signal via selected proteolysis of AUX/IAA proteins. Auxin facilitates the interaction between the AUX/IAAs and an SCF E3 ubiquitin ligase. SCF-type E3s are the most abundant type of ubiquitin ligase in Arabidopsis and several other signaling pathways including those for jasmonic acid, gibberellic acid, and the circadian clock are mediated by SCF-mediated protein degradation. The SCF ubiquitin ligase is a multi-subunit protein complex consisting of four subunits: SKP1 (ASK1 in Arabidopsis), a Cullin family member such as CUL1, a RING protein RBX1, and a target recognizing F-box protein. Here, we report the characterization of a novel, recessive allele of CUL1, cul1-6, isolated from a genetic screen in Arabidopsis thaliana (Col) designed to identify plants defective in degradation of a fusion protein with an Aux/IAA degron, IAA1:LUC. One mutation was identified that exhibited very slow IAA1:LUC degradation compared to the 10-12 min half-life of IAA1:LUC in the progenitor line as measured in cycloheximide chases. Genetic mapping placed the mutation within a 0.8 cM genetic interval that includes the CULLIN1 gene. We sequenced the coding region, 5' UTR, and 3'UTR of CUL1 and identified a mutation. Aerial portions of cul1-6 exhibit reduced apical dominance, delayed senescence, reduced fertility, and small, wrinkled rosette leaves. In root growth assays, the mutant roots were shorter, had fewer lateral roots than wild type, and were less responsive to 2,4-D. Genetic complementation by expressing FLAG-tagged wild type CUL1 under control of its endogenous promoter confirmed that this locus was responsible for the mutant phenotype. Mutant alleles of CUL1 have been isolated previously (Development 127:23-32; Plant J 43:371-83), but the mutations are all at the N-terminus and affect binding to the SCF subunit SKP1. No alleles that affect subunit interactions at the CUL1 C-terminus have been described to date. We believe the location of the mutation within CUL1 and its recessive nature will allow this allele to be used as tool to dissect regulation of SCF activity at the C-terminus of CUL1. This work was supported in part by National Science Foundation IBN 0212659 to JC.

386 SPINDLY-Dependant, DELLA-Independent Gibberellin Signaling Pathway to Suppress Cytokinin Responses

Yaarit Greenboim-Wainberg, David Weiss

The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel

We have shown previously that SPINDLY (SPY), a negative regulator of GA (gibberellin) signaling, promotes cytokinin responses in Arabidopsis. In addition, GA also represses the effects of cytokinin, suggesting that there is cross talk between the two hormone-response pathways and that this crosstalk requires SPY function. We demonstrated that SPY acts as both a repressor of GA responses and a positive regulator of cytokinin signaling and suggested that GA suppresses cytokinin responses through the inhibition of SPY. The DELLA proteins play a key role in the regulation of GA-signal transduction and in the interactions between GA and auxin, ethylene or ABA response pathways. Here, we determine whether the DELLA proteins also regulate the interaction between the gibberellin and cytokinin signaling pathways. GA-regulation of inflorescence-stem elongation depends on DELLA (GAI and RGA) protein activities and GA inhibits the suppressed inflorescence stem elongation after application of cytokinin. Cytokinin may arrest elongation by increasing the stability of the DELLA proteins, and GA may inhibit this effect by promoting the latter's degradation. Our results show that cytokinin has no effect on RGA stability or on GA-induced RGA degradation. Furthermore, the inhibition of stem elongation by cytokinin was suppressed in *spy* plants but not by the loss of GAI and RGA activities, suggesting that the GA signal generated by the loss of these DELLA proteins does not suppress cytokinin responses. Cytokinin also inhibited inflorescence elongation in the dwarf, gain-of-function *gai*. While GA's effect on stem elongation was completely blocked in *gai* plants, GA could still act in the mutant's stem to inhibit cytokinin-suppressed elongation. We suggest that the GA signal to suppress cytokinin responses is mediated by SPY but not by the DELLA proteins, and therefore it is possible that SPY acts in a DELLA-independent GA signaling pathway.

387 Molecular Functions of ARL2 and ARG1 in Arabidopsis Gravitropism

Benjamin Harrison, Changhui Guan, Patrick Masson

University of Wisconsin - Madison

Root gravitropism is an important model for studying signal transduction as well as fundamental aspects of plant growth and behavior. Root gravitropism involves the sensing of the gravity vector within the columella cells of the root cap, transduction of that information into a biochemical signal, transmission of that signal to the cells in the elongation zone, and a differential growth response.

ARG1 and its paralog ARL2 are mediators of early gravity signaling in Arabidopsis roots and hypocotyls. Mutations in either gene specifically result in reduced kinetics of gravitropic bending in both organs. Analysis of arg1 mutants indicates that ARG1 is active in gravity-sensing cells, where it is required for the fast and transient alkalinization of the cytoplasm upon gravity-stimulation (1). In addition ARG1 and ARL2 are required for the development of a lateral gradient of auxin across the root cap of gravity-stimulated roots (1 and herein). The development of a lateral auxin gradient following gravity stimulation appears to be essential for gravitropism and is thought to be mediated by the relocalization of PIN3 within gravity-sensing cells (2). We show that both ARG1 and ARL2 are required for relocalization of PIN3 to the new bottom of gravity-stimulated columella cells. We also demonstrate that mutations in ARG1 eliminate the contribution of PIN3 to the gravitropic response.

ARG1 and ARL2 encode DnaJ-like proteins, and along with another member (ARL1), comprise a small gene family in Arabidopsis. DnaJ proteins are known to interact with DnaK proteins and through this interaction modify the folding, activity, or localization of other target proteins. Sub-cellular localization of an ARL2-GFP fusion is very similar to ARG1 fusions indicating that they are both associated with several cellular membranes throughout the vesicle trafficking pathway (1 and herein).

In order to investigate the molecular function of ARG1 and ARL2 we have sought to identify physical interactors via yeast 2 hybrid. We present the identification and characterization of a protein which interacts strongly with ARL2 in yeast. Further characterization of this and other related proteins in Arabidopsis will help define the molecular mechanisms of ARG1 and ARL2 function.

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388 Characterization of a Herbivore-Inducible Arabidopsis Terpene Synthase Responsible for the Formation of the Volatile Homoterpene TMTT

Marco Herde[1], Katrin Gaertner[1], Iris Camehl[1], Benjamin Fode[1], Dorothea Tholl[2], Jonathan Gershenzon[3], Christiane Gatz[1]

[1]General and Developmental Plant Physiology, Georg-August-University Goettingen, Germany, [2]Department of Biological Sciences, Virginia Tech University, Blacksburg, Virginia, United States, [3]Department of Biochemistry, Max-Planck-Institute for Chemical Ecology, Jena, Germany

Volatiles play an important role in plant-plant and plant-insect interactions. A major component of the A. thaliana volatile blend is the C16 homoterpene TMTT (4,8,12-trimethyltrideca-1,3,7,11-tetraene). TPS28 is as a key enzyme in the biosynthesis of the TMTT precursor geranyl-linalool as revealed by the analysis of knock-out and overexpressing plants. Consitutive expression of TPS28 leads to retarded growth and lesions on the first cotyledons indicating fitness costs at this early developmental stage. TPS28 is constitutively expressed in parts of the flowers and siliques. In vegetative tissues, TPS28 transcription is induced by the fungal elicitor alamethicin, by feeding of P. xylostella larvae, and by the coronatine derivative coronalon. Induction requires an octadecanoid-derived signal as well as the F-box protein COI1. In addition, TPS28 is induced by wounding. Remarkably, the wounding response occurs even in the absence of COI1. It has been suggested that COI1 is involved in shuttling of yet unknown negative regulators to the proteasome. Inhibition of protein biosynthesis by cycloheximide led to TPS28 transcription indicating that inhibition of the synthesis of a labile repressor might be sufficient for induction. Interestingly, induction by cycloheximide requires COI1, indicating that COI1 is involved in the basal turnover of the postulated negative regulator. Our model suggests an accelerated degradation of the repressor after activation of the octadecanoid pathway. In order to identify this labile repressor a screen for promoter-up mutants was established using chimeric TPS28::luciferase and TPS28:: phosphinotrycin acetyl transferase constructs. Promoter deletion analysis using GUS as a reporter has shown that 300 bps upstream of the putative transcription start site are sufficient for the induction by cycloheximide, alamethicin and wounding. Within this minimal promoter we identified 62 bps which are necessary for induction with cyloheximide and alamethicin. Investigation of putative cis elements is in progress. These will be used to identify regulatory proteins using the yeast-one-hybrid approach.

389 Evolutionary Proteomics Identifies Ligand-binding Amino Acids Of The Cytokinin Receptor CHASE Domain

Alexander Heyl[1], Klaas Wulfetange[1], Birgit Pils[2], Nicola Nielsen[1], Georgy Romanov[3], Thomas Schmulling[1]

[1]Free University Berlin, Germany, [2]Julius Maximilian University, Wurzburg, Germany, [3] Russian Academy of Sciences, Moscow, Russia

In *Arabidopsis thaliana,* the plant hormone cytokinin is sensed by three cytokinin receptors (AHK2, AHK3, CRE1/AHK4). Using a bacterial binding assay, we show that the ligand is bound by an extracellular domain, the so-called CHASE domain (cyclases, histidine kinase associated sensory extracellular). This domain is not only found in plants, but also in many uncharacterized receptors of bacteria and lower eukaryots. Using the wealth of the available genomic sequence information, an modified evolutionary proteomics approach was taken to discover amino acids important for the cytokinin binding. We searched for residues which are conserved in the plant cytokinin receptors, but not in receptors binding other ligands and compared the differences in the evolutionary rates of the respective amino acids. Five amino acids within the CHASE domains of plants seemingly important for cytokinin binding were identified. Site-directed mutagenesis and *in vivo* binding assays confirmed the importance of four of the five selected residures. This study clearly shows the power of this novel approach in which the computational analyis of the genomic sequence information is used to generate an experimental verifyable hypothesis.

390 A CBL-Interacting Protein Kinase Is Involved in Early Nitrate Signaling

Heng-Cheng Hu[1, 2], Yi-Fang Tsay[1]

[1]Institute of Molecular Biology, Academia Sinica, Taiwan., [2]Institute of Genetics, National Yang-Ming University, Taiwan.

CHL1 is a dual-affinity root nitrate transporter in *Arabidopsis*. To elucidate the physiological impacts of CHL1, microarray using Affymetrix ATH1 indicated that CHL1 plays important roles in regulating several metabolism pathways, including N-assimilation, glycolysis, trehalose-6-phosphate pathway and some ion transporters. Interestly, some calcineurin B-like protein (CBL) and CBL-interacting protein kinase (CIPK) genes are up- or down-regulated in *chl1* mutant. The CBL-CIPK networks are well-known to be involved in stress signaling. Here we report that a CIPK is also involved in early nitrate signaling. Analysis of two independent knockout mutants indicated that CIPK can positively regulate the nitrate-induction levels of nitrate transporter genes and nitrate assimilation genes. Similarly, nitrate in the medium will inhibit the primary root growth of wild type, but the inhibitory effect of nitrate is eliminated in the *cipk* mutants. In conclusion, the CIPK is the first kinase found to be involved in nitrate signaling. Our data also indicated that in addition to stress, CBL-CIPK is also responsible for nitrate sensing.

391 Characterization of the Sugar-Insensitive Mutants *sis3, sis7* and *sis8*

Yadong Huang, Sue Gibson
University of Minnesota, Department of Plant Biology

Plants respond to environmental cues during seed germination and early seedling development. Sugar molecules, as well as phytohormones, coordinate to control seed germination and seedling growth. It has been shown by our lab and others that low to moderate concentrations of exogenous sugars (e.g. 30 mM sucrose) can delay seed germination in wild-type Arabidopsis. High concentrations of sugars (e.g. 300 mM sucrose) in the media inhibit cotyledon expansion and true leaf formation in wild-type seedlings. Several sugar insensitive (*sis*) mutants have been identified. Some of our recent results on three of these mutants, *sis7, sis3* and *sis8*, are presented here. The *SIS7* gene was recently identified using a map-based cloning approach and found to be the same as the *NCED3* gene, which encodes 9-cis-epoxycarotenoid dioxygenase, a key enzyme in the biosynthesis of abscisic acid. The *sis7-1* mutant exhibits resistance to mannose and to the gibberellin biosynthesis inhibitor paclobutrazol during seed germination. Genome wide transcriptional analyses of the *sis7-1* and *sis7-2* mutants and several other *sis* mutants have been performed and the results of these analyses will be described. The *sis3* mutant shows wild type or near wild type responses in all phytohormone response assays conducted to date. Another *sis* mutant, *sis8*, exhibits hypersensitivity to mannose and paclobutrazol. In addition, the morphology of *sis8* is distinct from the wild type. Identification of the *SIS3* and *SIS8* genes is in progress.

392 Arabidopsis Photomorphogenic Regulatory Network Discovery

Matthew Hudson, Kankshita Swaminathan, Stephanie Bellendir
University of Illinois, Urbana-Champaign

The transition between heterotrophic and autotrophic growth is orchestrated by a transcriptional regulatory network under the control of photoreceptors. While immediate / early events that transduce signals from the phytochrome photoreceptors to gene expression have been well studied, our understanding of the downstream transcriptional network is limited. Using our own computational tools, we have identified new cis-regulatory DNA motifs from Arabidopsis transcriptional profiling and genomic sequence data. We chose to focus on motifs specific to late responding light inducible promoters, since these later events may be mediated by unknown regulatory pathways. Four novel motifs were identified in the late responding, light inducible promoters. These motifs are capable of conferring light-inducibility on a reporter gene in vivo. To elucidate the transcriptional regulatory effects of the pathways that regulate these elements, we have constructed transgenic Arabidopsis lines that express the reporter gene luciferase under the control of these motifs. Results from these experiments will be presented. In addition, we have used the output of the motif discovery software, including the four late-response motifs, to develop a support vector machine model. Using this model, we are able to predict the behavior of a large number of *Arabidpsis* light-induced genes *in silico,* based solely on the sequence of their promoters. The annotation and prediction of gene expression from promoter sequence in Arabidopsis will be discussed.

393 The Protein-protein Interactions of the RCD1 Protein And Their Role in Plant Stress Signaling

Pinja Jaspers, Tiina Kuusela, Jaakko Kangasjarvi
Dept. of Biol. & Env. Sci, University of Helsinki

RCD1 (radical-induced cell death1) is an *Arabidopsis thaliana* protein whose function is essential in regulating reactive oxygen species-related signaling. The gene was originally identified through an ozone sensitive mutant *rcd1* that is not only sensitive to increased levels of ozone and superoxide but also has several alterations in its hormonal signaling. The *Arabidopsis* genome contains a close homolog of RCD1 called SRO1 and the characterization of these two proteins will be conducted in parallel.

There is no known biochemical function for RCD1 protein but it is thought to be localized to the nucleus and to have two domains involved in protein-protein interactions (WWE domain and a ”C-terminal domain”). In addition, RCD1 contains the catalytic core of ADP-ribosyl transferases and the protein sequence contains many potential post-translational modification sites. Available DNA microarray data suggests that the regulation on the RNA level is not strong and this, combined with the predictions of the protein structure, indicates post-translational regulation of the protein.

We have constructed a yeast 2-hybrid library and used it to search for interacting proteins to RCD1 and SRO1. Several interesting proteins were discovered (e.g. the transcription factor DREB2A) and the confirmation of these interactions *in planta* is ongoing. To elucidate the post-translational regulation of RCD1 we have studied its protein levels in wild type plants and in the *rcd1* mutant and *sro1* knock-out plants.

The information gained by the biochemical characterization of RCD1 will be combined with systemic biology approaches to gain insights to the regulation and transmission of plant stress signaling.

394 The role of a bZIP transcription factor in sugar signaling in Arabidopsis thaliana

Shin Gene Kang[1], John Price[1], Pei-Chi Lin[2], Jyan-Chyun Jang[1, 2]
[1]Plant Biotech Center and Department of Horticulture and Crop Science, Ohio State University, Columbus, OH 43210, [2]Plant Biotech Center and Department of Plant Cellular and Molecular Biology, Ohio State University, Columbus, OH 43210

Regulation of cell signaling can occur at many different levels. One such signaling mechanism is the interaction between DNA elements and DNA-binding transcription factors (TFs), which can act as a regulatory circuit to turn on or turn off gene expression. Despite the fact that at least 10% of all Arabidopsis genes are sugar responsive, very few regulatory circuits are known to be associated with sugar signaling. We hypothesize that sugar-responsive TFs play key roles in sugar signaling and TFs are likely control switches in an interconnected regulatory network. We have chosen a sugar-responsive bZIP transcription factor as a model to test this hypothesis. Gene expression analyses indicate that the bZIP is highly sensitive to sugar and sugar-repression of the bZIP requires hexokinase activity. Reverse genetic analyses indicate that the bZIP is involved in sugar-dependent growth responses. Because the bZIP knockout plants grow more vigorously than that of the WT on the sugar-free MS medium, we hypothesize that the bZIP may be involved in nutrient utilization. In addition, bZIP knockout plants are tolerant to the high salt that otherwise causes stunted root growth in the WT. Together these results suggest that the bZIP may work at a point where crosstalk between nutrient and stress signals takes place. To identify the upstream regulators of the bZIP, we have found several putative sugar responsive cis-regulatory elements in the promoters of bZIP and its co-expressed genes. To further understand the regulatory network, we will use ChIP-on-chip technique to identify downstream targets of the bZIP.

395 Functional analysis of phosphatidic acid during germination

Takeshi Katagiri, Kazuo Shinozaki

Plant Molecular Biology Laboratory, RIKEN Tsukuba Institute

Phosphatidic acid (PA) has been proposed to function as a lipid signaling molecule in plants. Physiological analysis showed that PA triggers early signal transduction events that lead to responses to abscisic acid (ABA) during seed germination. In a previous study, using PA-catabolic enzyme lipid phosphate phosphatase (LPP) knockout mutant, we measured PA production during seed germination and found increased PA levels during early germination (1). In this study, we focused on the PA-synthetic enzyme phospholipase D (PLD) and showed which PLD functions specifically on this process with each PLD T-DNA insertional mutant. The PLD knockout mutant showed ABA insensitive germination. This result confirmed that PA is involved in ABA signaling. Moreover, to determine several target genes downstream of PA, we performed microarray analysis on the PLD mutant during germination. We discuss about PA signaling pathway that involved in ABA signaling during germination.

(1) Katagiri et al., Plant J 43, 107-117, 2005

396 COG1, cogwheel in light signaling represses photoperiodic flowering time in Arabidopsis by an antagonistic interaction with GIGANTEA

Jeongsik Kim[1], Yumi Kim[1], Donha Park[2], Pyung Ok Lim[3], Miji Yeom[1], Hong Gil Nam[1]

[1]Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, Republic of Korea, [2]Department of Plant Biology/Plant Biotechnology Center, Ohio State University, Columbus, OH 43210, USA, [3]Faculty of Science Education, Cheju National University, 66 Jejudaehakno, Jeju-si, Jeju-do, 690-756, Republic of Korea.

Higher plants use day-length or photoperiod as an environmental cue to regulate many aspects of development including the transition from vegetative to reproductive stage. The control of flowering by day length is achieved by fine molecular regulations of CONSTANS (CO), a key molecule in control of photoperiodic flowering. GIGANTEA (GI) is largely believed to a major factor regulating CO transcripts in long days under circadian clock control, but understandings of its molecular mechanisms are largely unknown. *COG1*, cogwheel1 in light signaling, was reported to be a phytochrome-signaling component that acts as a light repressor of photomorphogensis in *Arabidopsis*. *cog1-1D* and *cog1-2D* also exhibited an extremely late flowering phenotype in long day condition, not in short day condition. In agreement with flowering phenotypes, the rhythmic expression of *CO* and *FT* in long-day condition was decreased. Based on genetic and molecular approaches, inhibition of flowering time by *COG1* in long days is not due to the aberrant clock function, but due to the direct repression of CO promoter activity. Taken together, COG1 directly interacted with GI in yeast and in mesophyll protoplast, supporting that COG1 controls photoperiodic flowering time by direct repression of CO transcripts through inhibitory interaction with GI directly.

397 A GPI-Anchored Protein Regulates Stimulus-Induced Root Hair Elongation

Yoshihiro Koshino-Kimura[1], Haruna Kiriyama[1], Akira Yoshimori[1], Miyuki Kubo[1], Takuji Wada[2], Taisuke Nishimura[3], Mayumi Ohta[1], Sumie Ishiguro[4], Ryuji Tsugeki[1], Noritaka Matsumoto[1], Kiyotaka Okada[1]

[1]Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan, [2]Plant Science Center, RIKEN, Yokohama 230-0045, Japan, [3]Laboratory of Plant Genetics, University of Geneva, Geneva, Switzerland, [4]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

To uptake water and nutrients efficiently, plants have many root hairs on their roots. In order to fulfill their function, roots and root hairs should touch soil. Length and direction of *Arabidopsis* root hairs are changed depending on touch or untouch of the root tip to agar surface. When roots are grown on agar surface, root hairs are perpendicular to the root surface, however, when roots come apart from the agar, hairs get longer and tilted to the root tip. To clarify the molecular mechanism of this response, we isolated mutants defective in this response. Root hairs of *timid* (*tmd*) mutant are shorter on agar than those of wild type, and become still much shorter when the root does not touch agar. The direction of the root hairs in *tmd* were, however, normally changed when the root came off the agar surface. Map-based cloning revealed that the *TMD* gene encodes a putative GPI-anchored protein. The *TMD* gene began to be expressed in hair-forming cells just before the root-hair bulge were observed. The TMD protein was shown to be localized in plasma membranes and endosomes. Possible function of TMD protein in the elongation of root hairs in response to the environment will be discussed.

398 Isolation and Characterization of SOR12, a Novel Regulator of Cytokinin-Mediated Leaf Senescence

In Chul Lee, Hyo Jung Kim, Tae Hoon Kim, Seung Hee Choi, Hong Gil Nam

Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, Korea

Senescence is a sequence of biochemical and physiological events that constitute the final stage of development. Senescence is now clearly regarded as a genetically programmed and evolutionally acquired developmental process. However, in spite of the biological and practical importance, genetic mechanism of senescence has been very limited.

Previously, we reported that *ore12-1* has increased leaf longevity due to a missense mutation in AHK3, a sensor histidine kinase cytokinin receptor, and suggested that cytokinins exert their anti-senescing effect specifically and positively through AHK3 to control senescence (Kim et al., 2006). To identify signaling components downstream of AHK3, we have undertaken a systematic genetic screening in an *ore12-1* allele through ethyl methanesulfonate (EMS)-mutagenesis. One suppressor named *sor12* (*suppressor of ore12-1*) was identified and showed complete suppression of the *ore12-1* senescence phenotypes. *sor12 ore12-1* double mutants exhibited accelerated senescence symptoms in age-dependent leaf senescence as well as in dark-induced senescence. Furthermore, *sor12 ore12-1* dramatically reduced the sensitivity of the plant to cytokinins in delaying leaf senescence and in inducing cytokinin-responsive genes, although these mutants still showed normal sensitivity to cytokinins in other responses, such as shoot induction and hypocotyl elongation inhibition.

Therefore, we suggest that SOR12 plays a major role in controlling cytokinin-mediated leaf senescence as a downstream component of AHK3. The identification of the mutated genes is underway and will be reported soon.

399 Identification and Characterization of Sugar-Response Genes by Genomics and Reverse Genetics in Arabidopsis

Chunyao Li, Tim Heisel, Sue Gibson
Department of Plant Biology, University of Minnesota

Sugars play a critical role in modulating plant development, metabolism and gene expression. In addition, sugar-response pathways are thought to help regulate allocation of fixed carbon between source and sink tissues, and so play an important role in carbon partitioning and, ultimately, crop yields. Sugar signaling networks are also found to interact with phytohormone signaling networks, such as those regulated by abscisic acid, ethylene and gibberellins. Despite the importance of sugar-response pathways, only a small percentage of the components predicted to act in these pathways have been identified so far. We are employing an approach combining genomics and reverse genetics to identify and characterize genes that are involved in sugar response pathways. By analyzing Affymetrix GeneChip data we selected 189 glucose- and/or sucrose-regulated genes that are predicted to encode proteins with activities commonly associated with response pathways, such as transcription factors, protein kinases and protein phosphatases. We then identified T-DNA insertion lines that are homozygous for insertions in most of these 189 target genes. Currently we are screening these T-DNA insertion lines for defects in any of several sugar responses. To date we have identified two mutants, *sov1* and *hac1*, that show altered sensitivity to the inhibitory effects of high concentrations of exogenous sugars on early seedling development. The *sov1* mutant exhibits a hypersensitive response to the inhibitory effects of both sucrose and glucose during seed germination and early seedling development. In addition, *sov1* is hypersensitive to the phytohormone abscisic acid. The *hac1* mutant exhibits weak insensitivity to sucrose during seed germination and early seedling development. In addition to *sov1* and *hac1*, we have also found that several other mutants exhibit alterations in sugar-regulated gene expression by real-time PCR analysis.

400 Light regulation of gene expression in soybean

Ying Li, Matthew Hudson
University of Illinois at Urbana-Champaign

Photomorphogenesis is relatively well understood in model systems like Arabidopsis. For example, the expression profiles of genes response to light signals in Arabidopsis have been examined in detail, using oligonucleotide microarrays. However, although the importance of photomorphogenic effects on crop morphology, physiology and yield are undoubted, photomorphogenic effects in crop plants are under-researched. In our study, we focused on the transcriptional regulation of photomorphogenesis in soybean using microarrays and real-time PCR. We used the soybean cDNA microarray developed by the NSF Soybean Functional Genomics project to find genes whose expression changes rapidly in etiolated soybean seedlings in response to a pulse of far red light. We then used published Arabidopsis microarray data to translate existing knowledge of photomorphogenesis into a better understanding of the mechanism of light-regulated development and signal transduction in soybean, and the natural variation in this process between soybean and Arabidopsis. We computationally determined the orthologous relationships between the genes represented on the NSF soybean cDNA microarray and the Arabidopsis oligonucleotide array, and compared the phytochrome regulated networks in soybean and Arabidopsis. Our results show that although some of the light regulated genes in the soybean microarray have orthologs in Arabidopsis, many are unique to soybean, suggesting overlapping and yet distinct transcriptional networks controlling photomorphogenesis in the two plant species. Results from this analysis will be presented.

401 Auxin stimulates serine phosphorylation of enolase in Arabidopsis roots

Tingfang Yi, Gaofeng Dong, Zenglan Wang, Jiaxu Li

Department of Biochemistry and Molecular Biology, Mississippi State University, Mississippi State, MS 39762

Protein phosphorylation plays an important role in auxin signaling. In order to identify phosphoproteins regulated by IAA (indole-3-acetic acid), proteins from Arabidopsis roots were separated by two-dimensional gel electrophoresis, probed with phosphoserine specific antibodies, and identified by tandem mass spectrometry. Enolase was identified as one of the phosphoproteins stimulated by IAA. Knockout mutant of enlase exhibited defective root development. The phenotype of enolase mutant could be partially restored to the wild-type phenotype by application of exogenous IAA. Together, these results suggest that enolase may be critical in regulating auxin homeostasis.

402 SPY Intracellular Site of Action in the Regulation of GA and Cytokinin Responses

Inbar Maymon, David Weiss

The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel

The Arabidopsis SPINDLY (SPY) protein is a negative regulator of gibberellin (GA) responses. We have shown previously that *spy* inhibits cytokinin responses and suggested that the protein acts as a positive regulator of cytokinin signaling. The SPY protein exhibits significant similarity to animal TPR-containing serine and threonine-*O*-linked *N*-acetylglucosamine (*O*-GlcNAc) transferase (OGT) enzymes. Similar to animal OGTs, SPY is present in both the nucleus and cytosol. We found that SPY intracellular localization is not affected by GA or cytokinin. To assess whether SPY's cytosolic/nuclear localization is required for the regulation of GA or cytokinin responses, we generated transgenic Arabidopsis *spy-3* plants expressing a SPY-glucocorticoid receptor (GR) fusion or SPY with a nuclear export signal (NES) under the regulation of the CaMV-35S or *SPY* promoters. Transgenic *spy-3* plants expressing SPY-GR and SPY-NES (homozygous lines) were tested for complementation. All transgenic *spy-3* lines expressing SPY-NES exhibited wild type phenotypes (e.g., sensitivity to the GA-biosynthesis inhibitor paclobutrazol, leaf serration and dark leaves). Similarly, transgenic lines expressing SPY-GR exhibited a wild type phenotype without the addition of dexamethasone (DEX). Furthermore, both SPY-NES and SPY-GR (without DEX) lines, exhibited wild type sensitivity to cytokinin. Our preliminary results show that DEX treatment of the GR lines display partial *spy-3* phenotypes. These results suggest that SPY acts in the cytosol to regulate both GA and cytokinin responses.

403 Association and Localization of PP2C Phosphatase/Stress-Activated MAP Kinase Complexes in Plant Cells

Irute Meskiene, Vaiva Kazanaviciute, Alois Schweighofer
Max F. Perutz Laboratories, University of Vienna, Vienna Biocenter, Dr. Bohrgasse 9, A-1030 Vienna, Austria

Mitogen-activated protein kinase (MAPK) pathways are commonly used to transmit environmental signals and integrate cellular responses in eukaryotes. Specific classes of protein phosphatases are employed to inactivate MAPKs, thereby influencing MAPKs activation kinetics (1). PP2Cs are ubiquitous protein phosphatases found in all eukaryotes, where they participate in a large array of signaling pathways. This diversity of biological functions is reflected in the great expansion of the PP2Cs family in Arabidopsis (2). We show by complementary approaches that AP2C1, a member of the Arabidopsis PP2C family, can specifically interact with and inactivate the stress-responsive MAPKs.

Direct protein-protein interaction in yeast and plant cells suggest high specificity in association between MAP kinases and phosphatase. We found that specific kinase interaction motif (KIM) identified *in silico* in AP2C1 is responsible for protein-protein interaction with substrate kinases. A similar KIM is found in yeast and animal MAPK interacting proteins, such as MAPKKs or MAPK phosphatases, as well as in plant MAPKKs.

Localization of *AP2C1* induction in transgenic *AP2C1::GUS* plants upon biotic and abiotic stress additionally supports its role in control of stress-activated MAPK. We show that AP2C1 and MAPKs associate into a complex in the nucleus or nucleus/cytoplasm, dependent on the MAPK involved by application of a bimolecular fluorescence complementation (BiFC) assay based on split-YFP (3).

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404 Interactions Between Light and Auxin Signaling Networks

Jennifer Moon, Hui Shen, Ling Zhu, Nidhi Sharma, Enamul Huq
University of Texas-Austin, Austin, TX 78712

Plant growth and development is modulated by the cues plants receive from their environment. Many individual signaling pathways, such as light or auxin signaling, have been extensively investigated. However, very little is known about how these signaling pathways are integrated to optimize plant development. We are interested in understanding the mechanism(s) of light and auxin signal integration. Previously, genetic and physical interaction has been shown between phytochrome (phy) light receptors and AUX/IAA proteins, transcriptional regulators of auxin response genes. To investigate whether the stability of AUX/IAA proteins is regulated by light signaling, we used full-length IAA3 or IAA4 fused to firefly Luciferase (LUC) driven by a strong Cauliflower mosaic virus (CaMV35S) promoter in transient transfection assays in Arabidopsis seedlings. We found that the luciferase activity of LUC-IAA3 and LUC-IAA4 was strongly reduced in red (Rc) and far red (FRc) light compared to the dark control, while the LUC-GFP control remained unchanged in all light conditions. Further, seedlings expressing only domains I and II of AUX/IAA17 fused to GUS (IAA17NT-GUS) show reduced GUS activity in Rc and FRc compared to the dark control, suggesting light signaling, like auxin signaling, may promote AUX/IAA degradation through domain II. Plants expressing GUS driven by an auxin response promoter, BA3, show reduced GUS activity in Rc and FRc indicating reduced auxin level/signaling in these light conditions. These results suggest that phy signaling modulates the abundance of AUX/IAA proteins directly or indirectly to control seedling deetiolation in response to light, and thus suggests a potential molecular mechanism for interactions between the light and auxin signaling pathways.

405 Nuclear-localized Phytochrome B Can Replace Phytochrome A Functions During Arabidopsis Seedling Photomorphogenesis

Candace Moore[1], Tomonao Matsushita[2, 3], Nathan Miller[1], Akira Nagatani[2], Edgar Spalding[1]
[1]UW-Madison, [2]Kyoto University, [3]UCLA

Phytochromes are red/far-red plant photorecepters that mediate aspects of seedling photomorphogenesis including hypocotyl growth inhibition and apical hook opening. Phytochrome A has been shown to mediate growth inhibition during the first three hours of red light, then a coordinated transition to a phytochrome B-dependent mechanism occurs. Our present research asks how nuclear localization of the PHYB protein affects the timing of the different phases of hypocotyl growth inhibition and hook opening. Using a novel high-resolution morphometric technique, the responses to red light of *phyAphyB* double mutant seedlings expressing either PHYB-GFP or PHYB-GFP containing a nuclear localization signal were investigated. We found that PHYA mediated the first 12 hours of growth inhibition induced by 10 μmol m^{-2} s^{-1} red light. Targeting PHYB to the nucleus compensated for PHYA action at this fluence rate and at the higher fluence rate of 50 μmol m^{-2} s^{-1}. Hook opening was also found to be PHYA-mediated at 10 μmol m^{-2} s^{-1}, and nuclear-localized PHYB also replaced this PHYA function. At the higher fluence rate of 50 μmol m^{-2} s^{-1}, hook opening was mediated largely by PHYB. Targeting PHYB to the nucleus increased the rate of hook opening in this condition. These data indicate that photomorphogenic responses at low fluence rates of red light are primarily mediated by PHYA while those at high fluence rates are mediated by both PHYA and PHYB. Nuclear targeting of PHYB can compensate for the lack of PHYA and speed up PHYB-dependent processes.

406 Genetic Interactions Between Brassinosteroid-Inactivating Enzymes and Photomorphogenic Photoreceptors

Katy Tiefenbrun, Leeann Thornton, Michael Neff
Washington University, St. Louis, MO

Activation tagging, a gene-overexpression mutagenesis tool, has been used to identify extragenic suppressors of the long-hypocotyl phenotype conferred by the photoreceptor mis-sense mutation phyB-4. Seven of these sob-D mutants (suppressor of phyB- dominant) have been identified and cloned in the Neff lab to date, some of which implicate cross talk between various hormone signaling pathways and photomorphogenic development. BAS1 and SOB7 encode a pair of cytochrome P450 enzymes that inactivate the growth-promoting brassinosteroid hormones. We generated single and double null-mutants of BAS1 and SOB7 to test the hypothesis that these two genes modulate photomorphogenesis. BAS1 and SOB7 act redundantly or synergistically with respect to light-mediated hypocotyl elongation inhibition and flowering time, photomorphogenic processes regulated by the photoreceptors phytochrome A (phyA), phytochrome B (phyB) and cryptochrome 1 (cry1). To test the hypothesis that P450-mediated brassinosteroid inactivation interacts with one or more of these photoreceptor-signaling pathways, we generated double-, triple- and quadruple-null mutant combinations between the null alleles bas1-2, sob7-1, phyA-211, phyB-9 and cry1-103. BAS1 and SOB7 act independently from phyB and cry1 to modulate hypocotyl growth in response to white, red and blue light. However, in far-red light the hypocotyl growth phenotype conferred by the loss of BAS1 and SOB7 requires phyA, demonstrating a role for this photoreceptor in modulating brassinosteroid inactivation. With respect to flowering, the bas1-2 sob7-1 double mutant and the phyB-9 single mutant flowers four leaves earlier than the wild type in long-day growth conditions (8 hrs darkness/16 hrs light). However these phenotypes are not additive since the phyB-9 bas1-2 sob7-1 triple mutant confers the same flowering phenotype as phyB-9 and the bas1-2 sob7-1 double mutant. The phyA-211 mutant flowers nine leaves later than the wild type in long-day growth conditions whereas the phyA-211 bas1-2 sob7-1 triple mutant flowers the same as the wild type. Surprisingly, the phyA-211 sob7-1 double mutant flowers four leaves later than phyA-211 even though the loss of SOB7 confers an early flowering phenotype in a bas1-2 null background. In contrast, the phyA-211 bas1-2 mutant flowers ten leaves earlier than phyA-211. In all cases, the flowering time data are supported by the number of days to bolting after germination. Together, these data demonstrate a complex interaction between phytochrome photoreceptors and P450-mediated brassinosteroid inactivation with regard to flowering time.

407 Functional Requirements for PIF3 in the De-etiolation Response

Bassem Al-Sady[1], Weimin Ni[1], Stefan Kircher[2], Eberhard Schafer[2], Peter Quail[1]

[1]Department of Plant and Microbial Biology, U. C. Berkeley, CA, 94720 and USDA/ARS, Plant Gene Expression Center, 800 Buchanan Street, Albany, CA 94710, USA, [2]Albert-Ludwigs-Universitat Freiburg, Institut fur Biologie II/Botanik, Schanzlestrasse 1, 79104 Freiburg, Germany

In order to gain an understanding of the mechanisms of phytochrome (phy) signaling, our laboratory has used yeast two-hybrid screens and other assays to identify proteins that interact directly and specifically with the biologically active Pfr form of the phy molecule. Our current focus is on defining the functional role and mechanism of action of phytochrome-interacting factor 3 (PIF3), a previously identified bHLH transcriptional regulator, and other closely related PIFs in the bHLH family. Reverse-genetic disruption of these loci indicates that each of the PIFs thus far examined appears to have a differential role in regulating phy-induced seedling deetiolation and, that PIF3 specifcally, is involved positively in controlling early phy-mediated gene expression and acts negatively, under prolonged red-light irradiation, on the phy-mediated hypocotyl growth response.

In trying to uncover the role of the interaction of PIF3 with photoactivated phys and with its G-box DNA target site towards PIF3's in vivo function, we have sought to specifically disrupt these interactions by targeted mutagenesis in the PIF3 protein. We have been able to dissect phyA and phyB binding sites in PIF3 and found that they are located in separate and unrelated domains of the PIF3 protein. In vivo mutant rescue analysis indicates that the requirements for phy and DNA binding towards PIF3's in vivo function can be temporally separated. We have further been interested in understanding how phys control the observed rapid light induced PIF3 degradation. Recent evidence indicates that light induces rapid phy-dependent phosphorylation of the PIF3 protein in vivo as a prelude to proteosomal degradation. This finding may provide insight into the biochemical mechanism of phy signal transfer to target proteins in the cell.

408 Evidence for Functional Conservation, Sufficiency and Proteolytic Processing of the CLAVATA3 CLE Domain

Jun Ni, Steven Clark

Department of Molecular, Cellular and Developmental Biology, University of Michigan

Members of the CLE (CLV3/ESR-related) protein family are small proteins broadly present in land plants. This family is defined by their similarity to Arabidopsis CLAVATA3 (CLV3) C-terminal sequence, a conserved domain termed CLE. This motif is also shared by several parasitism proteins from plant nematode species. Other than the CLE domain, CLV3 and the CLE proteins are not related in the rest of the proteins. CLV3 is a secreted protein. It has been hypothesized to act as a ligand for the CLV1/CLV2 receptor complex in the regulation of stem cell specification at shoot and flower meristems. Mutations within the CLE domain can disrupt CLV3 function. We have tested the ability of 13 Arabidopsis CLEs to replace CLV3 *in vivo* and found a significant variability, ranging from complete to no complementation. The best rescuing CLE depends on CLV1 for function, while other CLEs act independently of CLV1. Domain-swap experiments indicate that differences in function can be traced to the CLE domain within these proteins. Indeed, when the CLE domain of CLV3 is placed downstream of an unrelated signal sequence, it is capable of fully replacing CLV3 function. Interestingly, we have detected proteolytic activity in extracts from cauliflower (*Brassica oleracea*), Arabidopsis and tobacco that process CLV3, CLE1 and other CLE-containing proteins at their C termini. For CLV3, processing appears to occur at the absolutely conserved arginine-70 located at the beginning of the CLE domain. We propose that CLV3 and the other CLE proteins are C-terminally processed to generate an active CLE peptide.

409 The circadian clock and light signaling converge on bHLH transcriptional regulators to control rhythmic hypocotyl growth

Kazunari Nozue[1], Stacey Harmer[1], Michael Covington[1], Paula Duek[2], Severine Lorrain[2], Christian Fankhauser[2], Julin Maloof[1]

[1]Section of Plant Biology, College of Biological Sciences, University of California, Davis, [2]Center for Integrative Genomics, University of Lausanne, Switzerland

Most organisms use circadian oscillators to anticipate daily environmental changes, but little is known about how circadian systems interact with normal diurnal signals (1). Strikingly, we find that the growth phase of Arabidopsis hypocotyl in diurnal light conditions is shifted 8-12 hours relative to plants in continuous light, highlighting the importance of clock/environment interactions. Expression profiling by Affimetrix ATH1 genome array and functional analysis of various clock and photomorphogenic mutants revealed that two circadian regulated bHLH genes (PIF4 and PIL6) function as intermediaries between the clock and light signaling. This interaction explains the observed diurnal growth pattern and may serve as a paradigm for understanding intersections between endogenous and environmental control of other processes.

(1) Nozue and Maloof (2006) Plant Cell and Environment 26:396-408

410 Structure-Function Analysis of a Small Molecule that Alters Auxin-mediated Gene Expression in Arabidopsis thaliana

Sarah Miller[1], Johann Bergholz[1], Ronald Brisbois[2], Rebecca Hoye[2], Paul Overvoorde[3]

[1]Departments of Biology and Chemistry, Macalester College, St. Paul, MN 55105, [2]Department of Chemistry, Macalester College, St. Paul, MN 55105, [3]Department of Biology, Macalester College, St. Paul, MN 55105

A combination of biochemical and molecular-genetic approaches has recently provided insights into aspects of auxin-mediated gene expression. Despite these advances, additional components that regulate auxin-controlled processes or function in integrating multiple signaling pathways remain to be identified. Chemical genetics offers a powerful new approach to understand plant hormone action. Identification of small molecules that perturb a signaling pathway can lead to the isolation of the cellular targets of these compounds and their role in mediating signaling can be tested. This approach has previously been used to identify a number of small molecules that alter auxin-inducible expression of the BA3-GUS reporter gene (Armstrong et al., P.N.A.S. 101: 14978). As an outgrowth of efforts to provide interdisciplinary, research-based opportunities for our undergraduate students, we have initiated a structure-function analysis of compound A, a furyl acrylate ester of a thiadiazole heterocycle. Using the lab work of organic chemistry students as a starting point, we have synthesized fourteen analogs of compound A, and are currently characterizing the effects that each derivative has on auxin-regulated gene expression. Initially, the qualitative effect of each derivative is being determined by examining changes in auxin-inducible expression of DR5-GUS or BA3-GUS reporter genes. Based on these initial findings, quantitative real-time PCR will be used to define the effect of the derivatives on endogenous, auxin-modulated gene expression. These molecules will then be used in plate-based assays to monitor the effects that the molecules have on plant growth. Collectively, these data will provide insight into the active core component of compound A, which will aid in efforts aimed at identifying the cellular target(s) of this molecule.

411 Using High-throughput Chemical Genetics to Understand Abscisic Acid Hormonal Action

Jignasha Patel, Frederic Delmas, Peter McCourt

University of Toronto, Department of Botany

Abscisic acid (ABA) is a phytohormone that regulates many agronomical important aspects of plant development as well as mediating responses to stresses. The mechanisms by which ABA regulates these processes have yet to be fully delineated. A variety of approaches have been used thus far including genetic, biochemistry and cell biological. We are using a high-throughput chemical genetics approach to further understand ABA signaling. To begin, I have conducted two screens for chemicals that alter (enhance or suppress) the response of seeds (at the level of germination) to exogenous levels of ABA. To identify chemical enhancers of ABA, Arabidopsis seeds (Columbia ecotype) were screened on 4800 chemicals in the presence of 0.3 uM ABA for inhibition of germination. And to identify suppressors of ABA, seeds were screened with the same libraries of chemicals (LOPAC and Spectrum) on 3 uM ABA for seeds that were able to germinate. Chemicals with reproducible activities were further characterized as having ABA specific action by testing their effects with ABA for root growth and stomatal responses. Two chemical suppressors of ABA and seven chemical enhancers of ABA are currently being investigated.

In addition, low concentrations of ABA was found to have a protective effect against a group of chemicals that cause Arabidopsis seedlings to otherwise bleach. We hypothesize that ABA is acting as a chemical safener against these compounds and that it induces degradation or metabolism of the bleaching compound. HPLC analysis is currently being used to determine metabolic differences of these compounds observed in plants in the absence and presence of ABA.

412 IAR4 Modulates Basal Auxin Response Through Regulating Auxin Homeostasis

Marcel Quint[1], Lana Barkawi[2], Jerry Cohen[2], William Gray[1]

[1]Department of Plant Biology, University of Minnesota - Twin Cities, [2]Department of Horticultural Science, University of Minnesota - Twin Cities

In a screen for enhancers of tir1-1 auxin resistance, we identified two novel alleles of the putative mitochondrial pyruvate dehydrogenase E1alpha subunit, IAR4. The iar4-3 mutation interacts synergistically with tir1-1 in root growth inhibition and lateral root development assays. Additionally, iar4 single mutants exhibit numerous auxin-related phenotypes including auxin-resistant root growth and reduced lateral root development, as well as severe defects in primary root growth, root hair initiation and root hair elongation. To examine the effects of iar4 mutations on SCFTIR1 activity, the HS:AXR3NT-GUS reporter was introduced into iar4-3 to examine Aux/IAA protein stability. While the basal level and stability of the AXR3NT-GUS fusion protein are significantly increased in iar4-3 compared to wild-type, AXR3NT-GUS degradation in auxin-supplemented media is comparable to wild-type. Remarkably, all of the iar4 mutant defects are rescued when the seedlings are grown at high temperature (28C). Since auxin biosynthesis in planta is increased at high temperature, the iar4-3 phenotypes may be the result of a defect in auxin homeostasis. In support of this hypothesis, the activation-tagged allele of YUCCA, previously shown to confer elevated levels of free IAA, also rescues most of the iar4-3 mutant phenotypes. IAA measurements detected no significant difference between iar4-3 and wild-type for free IAA, but a significantly higher level of IAA-amino acid conjugates was observed in the iar4-3 mutant. We therefore suggest that iar4 mutations affect basal auxin responses via altered auxin homeostasis, perhaps due to localized or transient IAA dynamics not revealed by whole seedling analyses.

413 Arabidopsis EER4 encodes an EIN3-interacting TAFIID transcription factor that is required for proper response to ethylene, including induction of ERF1

Linda Robles, Jessica Wampole, Matthew Christians, Jesse Cancel, Paul Larsen
University of California-Riverside

Ethylene is a critical plant hormone that promotes the seedling triple response along with several agriculturally important phenomena including fruit ripening and tissue senescence. Through the identification of Arabidopsis mutants with either an ethylene insensitive or constitutive ethylene reponse phenotype, several components of the ethylene-signaling pathway have been uncovered, although large gaps in our understanding exist. Since it is unlikely that further screening for these mutant phenotypes will be profitable, novel approaches to identify new factors involved in this pathway are necessary. We have focused on identification of Arabidopsis mutants with enhanced ethylene responsiveness (eer), based on the assumption that these represent defects in factors required to dampen ethylene response. One of these mutants, eer4, has been extensively characterized, with this loss-of-function mutation resulting in extreme exaggeration of response to saturating levels of ethylene in the triple response assay. Molecular characterization of this mutant surprisingly revealed ethylene insensitivity with regard to induction of the ethylene-regulated genes AtEBP, basic chitinase, and ERF1, with the latter showing virtually no expression in eer4 leaves following ethylene treatment. Molecular cloning of the eer4 mutation showed that it represents an inappropriate stop codon in a previously uncharacterized TAFIID transcription factor that is ubiquitously expressed throughout the plant in an ethylene independent manner. Yeast two-hybrid and in vitro binding assays have indicated that EER4 strongly interacts with several known components of the ethylene signaling pathway including itself, CTR1, EIN3, and ERF1 along with the catalytic subunit of PP2a, PP2a1C. It was previously reported that loss of PP2a activity gives an identical eer phenotype, suggesting that dephosphorylation of EER4 is required for proper ethylene response. Based on our phenotypic, biochemical, and double mutant analyses, we propose that EER4 encodes a factor that associates with CTR1 in the absence of ethylene and then upon ethylene perception, it is dephosphorylated and transits to the nucleus where it recruits EIN3, ERF1, and likely other ethylene-related transcription factors for induction of genes such as ERF1 and AtEBP along with an as yet undefined group of genes required for resetting the ethylene response pathway following a signaling event.

414 PP2C type Phosphatase Regulates Stress-activated MAP Kinases

Alois Schweighofer, Vaiva Kazanaviciute, Irute Meskiene
Max F. Perutz Laboratories, University of Vienna, Vienna Biocenter, Dr. Bohrgasse 9, A-1030 Vienna, Austria

PP2C type phosphatases are important regulators of signaling pathways in eukaryotes. Plants, such as Arabidopsis contain the biggest family of PP2Cs suggesting that these phosphatases are significant in plant life processes (1). Here a new PP2C-type phosphatase from Arabidopsis was isolated and characterized. We identified its substrates as specific MAPKs (mitogen activated protein kinase) by yeast two-hybrid interaction approach. AP2C1 display exquisite substrate specificity in yeast and precisely down-regulates stress activated MAPKs in planta. Activation of these MAPKs by different cues inversely correlates to *AP2C1* expression in plants and isolated cells. Inactivation of stress MAPKs depends on the catalytic activity of AP2C1 phosphatase as indicated by introduction of loss-of-function mutation in catalytic part of the protein. Specific ability of this phosphatase to inactivate MAPKs is demonstrated in comparison with two other Arabidopsis PP2Cs, ABI2 and HAB1. Yeast two-hybrid screen of cDNA library with AP2C1 and analysis of knock out and transgenic plants lines over expressing this phosphatase supports its function on stress MAPKs and propose a model of AP2C1 action in plant cells that relates to MAPK activity control, pathogen response and ethylene production. Arabidopsis plants with altered AP2C1 contents display compromised innate immunity, modified wound responses (notably ethylene production) and perturbed expression patterns of defense-related genes. This is the first experimentally comprehensive report - at the molecular and whole-organism level - of a key role for a PP2C as a MAPK phosphatase in plants.

1. Schweighofer, A., Hirt, H. and Meskiene, I. (2004) Plant PP2C phosphatases: emerging functions in stress signaling. Trends Plant Sci, 9, 236-243.

415 The F-box Protein PPS Functions as a Positive Regulator of Light Signaling in Arabidopsis

Hui Shen, Phi Luong, Enamul Huq

Section of Molecular Cell and Developmental Biology and The Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712

Because light is vital for plants development, they are equipped with an array of photoreceptors that respond to different wavelengths of ambient light spectrum. Phytochromes (phys) and cryptochromes (crys) are the photoreceptors that respond to red (R), far-red (FR) and blue (B) regions of the spectrum by unknown mechanisms. Genetic approaches have identified both positive and negatively acting components in these light-signaling pathways; however, none of these pathways seem to be saturated. In an effort to identify additional components, we have screened available T-DNA insertional lines under continuous R light and identified a new mutant named *p*leiotropic *p*hotos*i*gnaling, *pps*. *pps* showed longer hypocotyls and smaller cotyledons under continuous R, FR and B light compared to that of the wild type. However, the long hypocotyl phenotype of *pps* is much stronger under R compared to FR and B. When grown in continuous white light, *pps* showed longer petiole length, higher number of inflorescence, shorter stature, and rounder leaves compared to that of the wild type. *pps* mutants are much smaller in stature and leaves of *pps* are much less expanded compared to that of the WT in short day (SD) conditions. Cloning of *PPS* using a combination of map-based cloning and candidate gene approach showed that it encodes MAX2/ORE9, an F box protein previously shown to be involved in inflorescence architecture and senescence. Since PPS is predicted to be a component of SCF complex involved in regulated proteolysis, these results suggest that SCF^{PPS} complex plays critical roles downstream of all light signaling pathways. In addition, these results also suggest that PPS may regulate multiple targets at different developmental stages to optimize plant growth and development.

416 Functional Analysis of the SAUR Family of Auxin-Inducible Genes

Angela Spartz[1], Star Weivoda[1], Hironori Ito[1], Paul Overvoorde[2], William Gray[1]

[1]University of MInnesota, St. Paul, MN, [2]Macalester College, St. Paul, MN

SAUR (Small Auxin Upregulated RNAs) genes were first identified nearly 20 years ago as primary auxin response genes. Arabidopsis contains at least 77 SAUR genes, which are predicted to encode low molecular weight proteins of 10-15kD. Sequence analysis of the SAUR proteins provides few clues as to their function, and what role if any they play in the auxin response pathway remains to be established. To investigate the functional importance of the SAUR genes, we have focused on a small subfamily containing SAURs 19-29, which share 73-95% identity at the amino acid level. Promoter-GUS reporter analysis has revealed overlapping, yet distinct, expression patterns. Notably, all of the promoter constructs tested are auxin inducible and exhibit strong expression in elongating cells of the hypocotyl. The large number of highly related SAUR genes suggests considerable functional redundancy in this gene family, complicating loss-of-function genetic studies. Therefore, we have taken an overexpression-based approach. Wild-type plants expressing a 35S:GFP-SAUR19 transgene exhibit several auxin-related phenotypes, including slight auxin-resistant root elongation, increased lateral and adventitious root development, elongated hypocotyls, and wavy root growth. Additionally, etiolated seedlings lack apical hooks and display increased root gravitropism and decreased hypocotyl phototropism. While these phenotypes are also observed with 35S:SAUR19 fusion proteins containing other N-terminal tags, plants expressing untagged SAUR19 appear completely normal. Preliminary immunoblot studies with a polyclonal SAUR19 antibody suggest that the N-terminal GFP tag stabilizes SAUR19, facilitating overexpression. We cannot however, rule out the possibility that the GFP-SAUR19 fusion protein acts in a dominant-negative manner. Nonetheless, these findings provide strong genetic support for the hypothesis that SAUR proteins play an important role in auxin response. We are seeking to elucidate the molecular basis for these phenotypes by identifying SAUR19 interacting proteins and through microarray analysis of our transgenic lines.

417 Calcium Entry Mediated by AtGLR3.3, a Plant Ionotropic Glutamate Receptor with a Broad Agonist Profile

Zhi Qi, Nicholas Stephens, Edgar Spalding
University of Wisconsin-Madison

Rapid, transient changes in cytosolic calcium couple endogenous and external signals to appropriate cellular response pathways in all manner of organisms. The molecular mechanisms that admit calcium across the plasma membrane of plants during signaling processes has remained elusive. Arabidopsis glutamate receptor-like (GLR) genes, homologous to ligand-gated ion channels of animal neuronal synapses, have been proposed as candidate genes for calcium entry into the cell, though direct experimental evidence for such a role has been lacking. Application of low (micromolar) concentrations of glutamate induces a large membrane depolarization and a concurrent, large rise in cytosolic calcium in wild-type root cells. Both responses depended on external calcium concentration, consistent with both being caused by calcium entering across the plasma membrane in response to glutamate treatment. Here we show that T-DNA insertions in the GLR3.3 gene (two independent alleles) completely block the depolarization and calcium transient induced by glutamate. An array of compounds was tested for GLR-agonist activity. The GLR3.3 ligand profile was found to include six structurally disparate amino acids (glu, gly, ala, cys, ser, and asn) as well as the tripeptide glutathione. Taken together, these results indicate a necessary role for this neuronal signaling homolog in amino acid recognition and calcium entry into the plant cell.

418 SOB3 and ESC are Two Plant-Specific Genes Involved in Seedling Development

Ian Street, Purvi Shah, Allison Smith, Michael Neff
Washington University, St. Louis, MO, USA

SOB3 was cloned from an activation tagging screen for suppressors of the long hypocotyl phenotype of a weak phyB allele, phyB-4. SOB3 encodes a single AT-hook containing protein with a second domain of unknown function and is part of a large and conserved plant specific gene family. sob3-D (suppressor of phyB-4, Dominant) overexpressing seedlings have shorter hypocotyls and as adults, develop significantly larger, rounder leaves, larger flowers and thicker stems than the wild type. The differences in organ size are due to cell proliferation as epidermal cell size is similar to the wild type. In addition, sob3-D plants have delayed senescence, living twice as long as the wild type. SOB3 is closely related at the DNA level to one gene in this family, ESCAROLLA, identified in a different activation tagging screen. esc-D plants are also suppressors of the phyB-4 hypocotyl phenotype and are similar as adults to sob3-D, suggesting that these genes are redundant with respect to over-expression. A loss-of-function SOB3 allele (sob3-4) was generated through an EMS intragenic suppressor screen of sob3-DphyB-4 plants. This allele induces a nonsense mutation in the transcript before either of the conserved domains. Two other alleles, both lesions in the AT-hook DNA binding domain were identified in this screen. These missense alleles suggest that the AT-hook DNA binding domain is important for SOB3 function. A similar nonsense allele of ESC (esc-8) was obtained from the Seattle TILLING Project. The sob3-4 esc-8 double mutant confers a long hypocotyl in multiple fluence rates of white and blue light. This double mutant and the esc-8 single mutant confer a long hypocotyl in far-red light when compared to the wild type or the sob3-4 single mutant. No significant differences were observed under multiple fluence rates of red light. In plants transformed with native promoter GUS translational fusion constructs of SOB3 or ESC, staining was observed in the seedling vasculature and in white light grown seedlings, throughout the hypocotyl. Dark grown seedling were stained mostly in the vasculature alone. In far-red light, GUS expression was observed predominantly in the hypocotyl. This suggests that SOB3 and ESC protein expression varies depending on the light condition. Taken together, these data suggest SOB3 and ESC can act redundantly to modulate hypocotyl growth inhibition in response to light.

419 Round The Clock – The Molecular System Of *At*GRP7

Corinna Streitner, Jan Schoening, Selahattin Danisman, Dorothee Staiger
Molecular Cell Physiology, Bielefeld University, D-33615 Bielefeld, Germany

The *Arabidopsis thaliana* glycine-rich RNA binding protein *At*GRP7 oscillates in a circadian manner with a peak in protein amount at the end of the day. Daily oscillation is initiated by rhythmic transcriptional activation of the *At*GRP7 gene by the circadian clock and fully developed via an autoregulatory feedback loop. In this negative feedback circuit the accumulation of the *At*GRP7 protein above a certain threshold is thought to lead to the emergence of an alternativly spliced *At*GRP7 mRNA containing a premature stop codon which prevents the translation of functional *At*GRP7 protein. Analysis of transgenic *Arabidopsis* lines constitutively overexpressing *At*GRP7 verifies this mechanism by showing the downregulation of the endogenous *At*GRP7 mRNA level and an increased amount of the alternate transcript. Band shift assays reveal sequences in the intron and in the 3'UTR as binding sequences for the protein. As the *At*GRP7 feedback curcuit operates downstream of the circadian clock, it may act as a slave oscillator passing circadian rhythmicity from the central oscillator to downstream targets. Microarray technologies are used to compare wild type plants with *At*GRP7 overexpressing plants and with RNAi-plants displaying an highly reduced level of *At*GRP7 expression in order to find target transcripts.

420 MEKK1 is a Negative Regulator of Stress Responses in Arabidopsis, but this Function does not Require the Protein's Kinase Activity

Maria Cristina Suarez[1], Lori Adams-Phillips[2], Shih-Heng Su[1], Peter Jester[1], Andrew Bent[2], Patrick Krysan[1]
[1]Horticulture Department and Genome Center of Wisconsin, University of Wisconsin - Madison, 1575 Linden Drive, Madison, WI 53706, USA, [2]Department of Plant Pathology, University of Wisconsin - Madison, Madison, WI 53706, USA.

The Arabidopsis gene MEKK1 encodes a MAP Kinase Kinase Kinase (MAP3K) that has previously been implicated in the regulation of biotic and abiotic stress response pathways. Here we report the characterization of two independent T-DNA mutant alleles of MEKK1 and demonstrate that plants homozygous for either of these mutations display a severe dwarf phenotype. Genome-wide expression analysis indicated that mekk1 plants constitutively express a battery of genes normally upregulated in response to biotic stress. The mekk1 dwarf phenotype could be rescued by transformation with an ectopic copy of the wild-type MEKK1 gene. More surprisingly, phenotypic rescue could also be achieved by the introduction of a kinase-inactive allele of MEKK1 (K361M) into mekk1 plants. These K361M plants were tested for their ability to transduce signals previously thought to require MEKK1 kinase activity, and it was found that K361M plants displayed wild-type responses to the elicitor peptide flg22 (a flagellin analog) as well as mechanical wounding. It was also observed that K361M plants exhibit only subtle changes in their response to virulent and avirulent Pseudomonas syringae. Our results indicate that MEKK1 acts as a negative regulator of stress response pathways in Arabidopsis, but this functionality does not require the protein's kinase activity. We propose a model for MEKK1 function in which the protein has a unique structural role in regulating stress response, but a dispensable kinase activity that may be compensated for by related MAP3Ks.

421 *Arabidopsis* Receptor-like Cytoplasmic Kinases Involved in Growth and Development

Carl-Erik Tornqvist, Ronan O'Malley, Jenny Liu, Anthony Bleecker, Sara Patterson
University of Wisconsin, Madison

Plant receptor-like kinases (RLKs) have been implicated in growth and development, disease resistance, stress response, and self-incompatibility. The *Arabidopsis* RLK gene family contains over 600 members, yet only a handful have known functions. Using a high-throughput reverse genetics approach, we have isolated homozygous T-DNA lines spanning 26 subfamilies and covering much of four. Specifically, we have focused on receptor-like cytoplasmic kinases (RLCKs), which lack the transmembrane and extracellular domains of typical RLKs. These proteins may function as intermediate signaling components between other kinase players. Such interactions have been shown in pathways of *Pseudomonas* resistance and self-incompatibility. All mutant lines have been screened for alterations in growth and development and responses to phytohormones, including: auxin, cytokinin, gibberellin, and ethylene.

Currently, only three single mutant lines and one double mutant display growth defects. All four mutant lines have disruptions in RLCKs; all from subfamily RLCK VII. Two single mutants have altered root and hypocotyl growth. A double homozygous mutant, which was generated between two closely related mutant lines with no phenotypes, appears to have delayed flowering, evidenced by much larger rosettes, thicker primary shoot, and longer time to bolting than wild type. Of particular interest is a single homozygous mutant we call *lacy*, which displays asymmetric leaf morphology, reduced leaf size, small stature, altered apical dominance, and irregular trichomes with supernumerary branching. We have conducted preliminary phenotypic characterizations of all four mutant lines, with most emphasis on the *lacy* mutant. To answer the question of why *lacy* has altered leaf development, epidermal cell size and cell number were compared to wild type. The pattern of cell division was also examined in the mutant and wild type by introgression of a construct harboring the Cyclin1a promoter with the GUS reporter (Ferreira, 1994). In addition to phenotypic characterization of *lacy*, genetic interactions with the most closely related RLKs were also analyzed. We will present the phenotypes of these four mutants, some expression data culled from microarray experiments, and summarize genotyping results for the entire homozygous set.

422 Functional Genomic Analysis of the 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE (ACS) Gene Family Members in Arabidopsis thaliana: Construction and Characterization of a pentuple Mutant

Atsunari Tsuchisaka[1], Hailing Jin[4], Jose Alonso[2, 3], Joseph Ecker[2], Athanasios Theologis[1]
[1]UCB/USDA Plant Gene Expression Center, Albany, CA 94710, USA, [2]Salk Institute for Biological Studies, La Jolla, CA 92037, USA, [3]Department of Genetics, North Carolina State University, Raleigh, NC 27695, USA, [4]Department of Plant Pathology, University of California at Riverside, CA 92521, USA

1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE (ACS) catalyzes the rate-limiting step in the ethylene biosynthetic pathway in plants. The Arabidopsis genome encodes nine ACS polypeptides that form eight functional (ACS2, ACS4-9, ACS11) and one non-functional (ACS1) homodimers. The ACS polypeptides have also the capacity to form active (17) and inactive (25) heterodimers. Herein, we report the identification and characterization of T-DNA insertion lines for five (ACS2, ACS4, ACS5, ACS6 and ACS9) among the nine ACS genes. All single insertion alleles show an enhanced plant size and the acs6-1, acs9-1 mutants flower earlier than the wild type plants. Subsequently, we generated 19 double, 25 triple, 16 quadruple, and 4 pentuple mutants, using various insertional alleles of the single T-DNA lines. There is a progressive enhancement in plant height in the higher order mutations. A pentuple mutant (acs2-1 acs4-1 acs5-2 acs6-1 acs9-1) was phenotypically characterized in a great detail and its phenotype was compared to those of two ethylene perception mutants, etr1-1 and ein2-5. The pentuple mutant forms a normal hook, flowers earlier and is taller than the wild type. The sizes of its siliques, flowers, seeds, leaves are the same as the wild type. Its responses to the infection with the bacteria pathogens, Xanthomonas campestris pv. vesicatoria (Xcv), Xanthomonas campestris pv. campestris (Xcc), necrotropic fungus Alternaria brassicicola and oil rape mosaic virus (ORMV) are the same as the wild type. Inactivation of the Arabidopsis ACS capacity by eighty percent in the pentuple mutant yields a perfectly functional plant and does not delay its senescence. These observations provide molecular insight into the unique and overlapping functions of the ACS gene family members in Arabidopsis. The pentuple mutant also provides the framework for future construction of a "Yang cycle"-dependent null ethylene producing Arabidopsis plant, to ascertain whether ethylene is required for plant growth and development.

423 Protein Prenylation Process Negatively Regulates Abscisic Acid Signaling on Seed Germination and Plant Drought Response in Arabidopsis thaliana

Xuejun Wang, Mark Running
Donald Danforth Plant Science Center

PLURIPETALA (*PLP)* encodes the α-subunit sharedbetween protein farnesyltransferase (PFT) and protein geranylgeranyltransferase-I (PGGT-I) in *Arabidopsisthaliana*. Knockout of PLP (*plp*) show dramatically larger meristem, increased floral organ number, ABA-sensitivity, and extreme drought tolerance. Knockout of the β-subunitof PFT (*ERA1*) show milder developmental phenotypes and drought tolerance, but stronger ABA-hypersensitivity in seed germination assays compared to *plp*. ABA response of stomataapertures of *ggb*, which is the knockout of PGGT-I β-subunit, were very mild in phenotype. To learn the interactions of *PLP* and GGB in ABA response, we tested the development, seed germination on ABA, drought stress response, and stomata aperture of single and double mutants of *plp* and ABA biosynthesis and response mutants. Phenotypes of *plp aba1-4, plp aba1-6, plp aba2-1,* and *plp aba3-1* show a synergistic developmental phenotype of much smaller plants, suggesting that PLP does not mediate all ABA responses and that not all PLP phenotypes depend on ABA. The double mutants also indicate that ABA is important for long term but not short term drought tolerance of *plp* plants. Double plants of *plp abi1-1, plp abi2-1, plp abi3-1, and plp abi4-101* showed same developmental phenotype as *plp* alone. *plp abi1-1* and *plp abi2-1* doubles were sensitive to ABA on seed germination, stomata aperture, and drought stress, while *plp abi3-1 and plp abi5-1* doubles were insensitive to ABA in these assays. Our result implicated that *ABI1* and *ABI2* act upstream of *PLP* and *ABI3* and *ABI5* act downstream of *PLP*. *plp abi4-101* double showed similar results as wild type in all assays, suggesting they act in parallel pathways. Interestingly, *abi5-1 plp* plants show a dramatic rescue of *plp* developmental phenotypes, suggesting that *abi5-1* acts downstream of *plp* in pathways other than ABA response.

Corresponding author: Running P. M. Email: mrunning@danforthcenter.org

424 Prephenate dehydratase 1 Activity is Critical for Protection from UV Radiation Damage in Etiolated Seedlings

Katherine Warpeha, Jack Gibbons, Andy Carol, Syed Lateef, Bob Lee, Lon Kaufman
University of Illinois at Chicago

Understanding the relationship between the synthesis of phenylalanine and the synthesis of UV screening pigments will become increasingly important as the levels of incident UV-B radiation increase. We recently demonstrated BL or ABA treatment of etiolated wt seedlings leads to the synthesis of phenylalanine via activation of a signal transduction mechanism comprised of GCR1, GPA1 and Prephenate Dehydratase 1 (PD1). The phenylalanine produced by PD1 in etiolated seedlings is used by the phenylpropanoid pathway to produce a range of UV absorbing compounds, none of which are produced in seedlings deficient in PD1, GPA1 or GCR1. Brief high energy radiation is lethal to etiolated *pd1*, *gcr1*, and *gpa1*, yet wt and *cry1cry2* and mutants in other members of the PD gene family (*pd3,4,5* & *6*) do not exhibit lodging in response to the same treatment. The cotyledons of etiolated wt and *pd1* mutants exhibit differences in the distribution of UV absorbing compounds following brief exposure to UV (366 nm) light. Because they are slow to accumulate chlorophyll and lack characteristic pink background autofluorescence, we examined the plastid status in etiolated *pd1* T-DNA insertion mutants. The cotyledons of etiolated *pd1* insertion mutants have a proplastid in contrast to etioplast present in wt seedlings. SEM data of etiolated wt seedlings demonstrates that a thick wax coat originates at the tip of the cotyledon in both untreated and UV treated seedlings. In contrast, *pd1* mutants do not accumulate this material regardless of the light treatment. Osmium tetroxide staining confirms that the cotyledons of dark-grown *pd1* insertion mutants have less cuticular surface wax and fewer long chain fatty acids, particularly at the very tip of the cotyledon. SEM data also indicate that the cotyledons of UV treated *pd1* mutants and not those of wt seedlings, accumulate waxy material on both the adaxial and the abaxial surfaces. We are interested in the determining the roles of the PD1-produced pigments and waxy materials in young etiolated seedlings, and how these PD-1-derived materials function in UV-screening and protection.

425 Characterization of an intragenic suppressor of *bri1-5*

Michael Wierzba[1], Rene Michel[1], Amanda Durbak[1], Robert Schmitz[2], Andrea Aguirre[1], Scott Rowe[3], Shozo Fujioka[4], Frans Tax[1]

[1]University of Arizona, Tucson, AZ, [2]University of Wisconsin, Madison, WI, [3]Washington University, St. Louis, MO, [4]RIKEN, Japan

The receptor-like kinase (RLK) Brassinosteroid Insenstive-1 (BRI1) is the major receptor for steroid hormones in Arabidopsis. Null mutants in BRI1 are dwarfed and display a reduction in the length of leaves, petioles, pedicels and internodes. Using the weak allele *bri1-5*, we isolated a dominant suppressor of its dwarf phenotype. The results of genetic and molecular tests indicated that the suppression was caused by a second site mutation in BRI1 that we named *bri1-11*. A thorough characterization of *bri1-5 bri1-11* and *bri1-5* phenotypes revealed that in most tissues, *bri1-5 bri1-11* plants were intermediate between *bri1-5* and wild type in size and in their response to BRs. These results suggested that the *bri1-11* mutation partially restored the function of *bri1-5*. To understand the mechanism through which the *bri1-5 bri1-11* receptor works, we manipulated the activity of ligand, the BRI1 interacting RLK BAK1, and the downstream kinase BIN2. Here we report these results as well as additional characterization.

426 Characterisation of New Regulators in ROS-Induced Cell Death

Michael Wrzaczek[1], Mikael Brosche[1], Hannes Kollist[1, 2], Triin Kollist[1, 2], Jaakko Kangasjarvi[1]

[1]Plant Biology, Department of Biological and Environmental Sciences, Viikki Biocenter, University of Helsinki, POB 65 (Viikinkaari 1), 00014 Helsinki, Finland, [2]Institute of Molecular and Cell biology, University of Tartu, Riia str. 23, Tartu, Estonia

Initially considered primarily as toxic compounds reactive oxygen species (ROS) are accepted to play a more complicated role. ROS appear to be ubiquitously used as signaling compounds that are produced in response to many biotic and abiotic stresses, including short and high pulses of the atmospheric pollutant ozone (O_3) and pathogen infection. The formation of ROS in the apoplastic space of leaves triggers hypersensitive response-like programmed cell death (PDC) lesions in the leaves of sensitive plants. This is a common feature of plant responses to ozone and pathogens and appears to be an active process during which ROS exhibit signaling functions in inter- and intracellular communication. While central components to pathogen recognition and signaling have been identified, the key elements in ROS perception as well as the regulators of lesion spread and lesion containment remain elusive. Receptor-like kinases (RLKs) exhibit important functions in the sensing of pathogens and developmental cues. Subsequently signaling networks are triggered including phosphorylation and de-phosphorylation events that lead to the adjustment of gene expression. Ultimately this leads to a co-ordinated and fine-tuned defense response. Taken together it is conceivable that ROS are perceived and processed in a similar way to pathogens, potentially sharing several signaling components.

Genes were selected for early transcriptional upregulation upon O_3 exposure. A screen for O_3 sensitivity in the corresponding knockouts identified several signaling components including amongst others components in calcium signaling, a putative lipase, various proteins of unknown function and two RLKs. Obtained data suggests the involvement of phosphorylation cascades in ROS signaling. Furthermore, the small extracellular proteins as well as RLKs might be directly involved in the regulation of O_3-induced lesion formation and possibly ROS perception. The study of the biological function and the biochemical properties of the identified proteins will allow for new insights into ROS perception and early ROS signaling. Furthermore these results will help to understand the role of ROS signaling in other biotic and abiotic stresses.

427 Integration of light and abscisic acid signaling during seed germination and seedling growth

Hao Chen, Liming Xiong
Donald Danforth Plant Sceince Center

The phytohormone abscisic acid (ABA) plays important roles in plant growth and development including seed dormancy, stomata movement, stress adaptation, and flowering. ABA also partially mediates drought rhizogenesis, a novel adaptive response of roots to drought stress. In this study we identified that HY5, a well-characterized component in the light signal transduction pathway, is also required for ABA-mediated drought rhizogenesis and ABA inhibition of seed germination and seedling growth. In hy5 mutant seeds the transcript levels of several ABA-regulated genes such as AtEM1, AtEM6, Rab18, and ABI5 were greatly reduced. Given that ABI5 has been shown to bind to AtEMs promoters and activate their transcription, we hypothesized that HY5 might be able to directly regulate ABI5 expression. Indeed, in vitro gel retardation assays showed that HY5 protein was able to bind to ABI5 promoter. This surprising discovery prompted us to investigate whether ABI5 has any role in light signal transduction. The ABI5 gene was then ectopically expressed in the wild type and *hy5* backgrounds. Overexpression of ABI5 in hy5 conferred increased ABA sensitivity to hy5. Furthermore, overexpression of ABI5 in the wild type resulted in enhanced light responses and shorter hypocotyls. Our data thus indicate that HY5 functions in ABA and light signaling partially through activating ABI5. Supported by NSF (grant #0521250) and USDA-NRI (grant #2005-35100-15275) (to L.X.)

428 Novel transcription factors involved in brassinosteroid signal transduction in Arabidopsis

Yanhai Yin[1], Michelle Guo[1], Li Li[1], Lei Li[1], Xiaofei Yu[1], Shigeo Yoshida[2], Tadao Asami[2], Joanne Chory[3]
[1]Department of Genetics, Development and Cell Biology, Plant Science Institute, Iowa State University, Ames, IA 50011, [2]Plant Functions Lab, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama 351-098, Japan, [3]Howard Hughes Medical Institute and Plant Biology Laboratory, The Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd., La Jolla, CA 92037

Plant steroid hormone brassinosteroids (BRs) play important roles throughout growth and development. Unlike animal steroid hormones that bind nuclear receptor superfamily transcription factors to directly regulate target gene expression, BRs are perceived by membrane-localized receptor kinase BRI1. Multidisciplinary approaches have been used to study how the BR signal is transduced from the cell surface receptor to nuclear target genes. By screening for bri1 mutant suppressors, we identified a nuclear protein BES1 that functions downstream of BR receptor to mediate target gene expression. Although BES1 does not have significant homology to known DNA-binding domains, its amino-terminal domain is involved in DNA binding and can potentially form a novel basic-helix-loop-helix (bHLH) motif. Indeed, BES1 is a transcription activator that binds to and activates BR-target gene promoters both in vitro and in vivo. In addition, BES1 interacts with a typical bHLH protein, BIM1, to synergistically bind to E-box sequences present in many BR-induced promoters. Our recent genetic, genomic and molecular studies identified several new transcription factors that cooperate with BES1 and regulate BR target gene expression. Loss-of-function and/or gain-of-function mutants of these BES1 partners display various BR-response phenotypes including changed resistance to BR biosynthesis inhibitor brassinazole. Our results therefore establish that BRs signal to regulate transcription activator BES1 and BES1 functions with other transcription factors to regulate plant growth and development.

429 Involvement of Phytohormone Signaling Pathways During Seed Germination Under Salt and Osmotic Stress Conditions

Kun Yuan, Joanna Diller
Department of Biological Sciences, College of Sciences and Mathematics, Auburn University

Soil salinity is one of the most significant abiotic stresses limiting plant growth. The adaptation of plant cells to stress conditions involves triggering a network of signaling events. The plant hormone abscisic acid (ABA) regulates many important aspects of plant growth and development, and plays a critical role in stress responses. The process of seed germination is affected by salt and osmotic stress at least partially via the ABA signaling pathway. Several components in the GA signaling pathway are known to be involved in germination but have not been tested for their involvement in salt and osmotic stress. We examined the responses of several mutants in GA signaling pathway to salt and osmotic stress during seed germination and early seedling development. Several mutants in the ABA signaling pathway, previously demonstrated to be involved in seed germination under these stress conditions, were used as positive controls. Real-time PCR was employed to test the genes relative expression levels in several mutants and under various stress conditions to determine whether salt and/or osmotic stress affects the seed germination via transcriptional control of the components in GA or ABA signaling pathway. This study suggested that different genes in ABA and GA signaling pathways are involved during different developmental stages under stress condition. We will also report on possible crosstalk between different hormone signaling pathways under the salt and osmotic stress conditions.

430 Investigating the Role of ETA2/CAND1 in Regulating SCF Complex Activity

Wenjing Zhang, Hironori Ito, Marcel Quint, William Gray
Department of Plant Biology, University of Minnesota-Twin Cities

The eta2-1 mutant was identified in a genetic screen for enhancers of the tir1-1 auxin (eta) response defect. eta2-1 plants exhibit several phenotypes related to impaired auxin response. Molecular studies found that these phenotypes are the result of reduced SCF-TIR1 activity in eta2-1 mutants (1). Isolation of the ETA2 gene revealed that it encodes an Arabidopsis ortholog of human CAND1 (Cullin-Associated and Neddylation-Dissociated). Biochemical studies with mammalian cell lines suggest that CAND1 acts as a negative regulator of SCF function by sequestering unmodified CUL1 away from SKP1 and the F-box protein, thus preventing assembly of the SCF complex. In contrast, we find that the eta2-1 mutation diminishes the ability of CAND1 to interact with CUL1, demonstrating that the interaction between these two proteins is required for SCF activity and that CAND1 positively regulates SCF function. These paradoxical findings have been explained by a model invoking CAND1 in regulating a dynamic cycle of assembly and disassembly of the SCF complex in vivo, through association and dissociation with CUL1. Double mutant analysis with the axr6-2 and eta1/axr6-3 alleles of CUL1 reveals additional insight into the interactions between CAND1 and CUL1. Whereas eta2-1 and axr6-3 interact synergistically, eta2-1 axr6-2 double mutants show mutual suppression of eta2-1 and axr6-2. Although the eta2-1 mutation itself is recessive, its suppression of axr6-2 is dominant, indicating a heightened sensitivity to CAND1 dosage level. Since the eta2-1 mutation dramatically reduces CUL1 binding activity, these genetic findings suggest that the axr6-2 mutation may inhibit dissociation of CUL1 from the CAND1-CUL1 complex. Consistent with this possibility, co-immunoprecipitation experiments detect a dramatic increase in CAND1-CUL1 complex abundance in axr6-2 mutant extracts in comparison to wild-type. Phenotypic and biochemical studies of these double mutants will be discussed in the poster. Molecular studies examining the effects of these mutations on SCF homeostasis are underway.

1. Chuang et al. (2004) Plant Cell 16, 1883-97

431 Functional analysis of ROP10 small GTPase-gated, low dose-specific genes in abscisic acid signalling

Zeyu Xin[1], Zhi-Liang Zheng[1, 2]

[1]Department of Biological Sciences, Lehman College, City University of New York, Bronx, NY 1046, [2]Plant Sciences PhD Program, Graduate School and University Center, City University of New York, New York, NY 10016

Abscisic acid (ABA) is a key hormone that modulates both agronomically important growth and development processes and responses to dynamic changes of biotic and abiotic environments, but our understanding of the ABA signal transduction network remains fragmented. ROP10, a member of bona fide signaling small GTPases in Arabidopsis thaliana, is a plasma membrane-associated protein specifically involved in the negative regulation of various ABA responses. To dissect the ROP10-regulated ABA signaling, we analyzed the transcriptome of the low ABA dose response. Together with molecular and bioinformatic analyses, we have revealed two groups of genes that are likely specific to low doses of ABA. The first group consists of 80 genes that are independent of ROP10 and the second group is gated by ROP10, including several regulatory genes such as receptor-like kinases and transcription factors. We now report the isolation and characterization of T-DNA knockout mutants for most of the ROP10-gated genes, and have found some of them altered ABA responses. The finding of ROP10-gated, low ABA dose-specific components in ABA signaling is novel. This implicates that plants use this mechanism to distinguish low versus high levels of ABA and/or mild versus severe magnitudes of stresses in order to fine tune the ABA and stress signaling network.

432 Ice Cap version 2.0: An Improved Method for High-Throughput Tissue Harvest and Genotyping in Arabidopsis

Katie Clark[1], Patrick Krysan[2]

[1]Department of Genetics, University of Wisconsin-Madison, Madison, WI 53706, USA, [2]Horticulture Department and Genome Center of Wisconsin, University of Wisconsin-Madison, Madison, WI 53706, USA

We previously developed a method named "Ice Cap" for growing Arabidopsis plants and harvesting tissue samples in 96-well format. The name of this method reflects the fact that root tissue is captured in 96-well plates by freezing the water in which the roots are growing. The seedlings from which the root tissue has been harvested remain viable in a second 96-well plate, thereby allowing individual plants with a desired genotype to be propagated. We have made several significant improvements to the Ice Cap procedure in order to make the process more robust. The cornerstone of Ice Cap 2.0 is a novel watering system that maintains a constant water level in the root growth plate. Without this watering system it can be difficult to achieve optimal seedling growth prior to the time when the water in the root capture plate dries out. The incorporation of this new watering system into the Ice Cap procedure also made it possible to grow the seedlings in 96-well plates with no lid covering the seedlings. This modification allowed improved air circulation throughout the plate, resulting in healthier seedlings. Details of the various technical improvements to Ice Cap will be described.

We have also developed an improved strategy for PCR-based genotyping of T-DNA insertion alleles as part of our streamlined genotyping pipeline. We will describe the details of a single-tube, multi-plex PCR method for determining if a plant is homozygous, heterozygous, or wild-type at a given locus. By using allele-specific primers carrying a generic 21-bp tag sequence, we are able to uniformly amplify PCR products in a single reaction from either or both of the two alleles that may be present. The resulting products are then analyzed by performing SYBR green-based melt curve analysis. The optimized protocol that we have developed constitutes a fast, simple, cheap, and accurate method for screening large numbers of Arabidopsis plants that are segregating T-DNA mutant alleles.

433 The Pyrabactins: Small Molecule Agonists of the Abscisic Acid Signaling Pathway

Pauline Fung, Freeman Chow, Yang Zhao, Nicholas Provart, Sean Cutler

University of Toronto, Department of Botany

We performed a chemical genetic screen of a 10,000 member small molecule library to identify compounds that disrupt hypocotyl cell expansion in etiolated Arabidopsis seedlings. Six compounds were identified that inhibit germination and ~750 compounds were found to reproducibly inhibit hypocotyl growth by >20%. Two of the germination inhibitors identified are structurally similar and examination of an analog series shows that a pyridine moiety is essential for the activity of these compounds that we have named the Pyrabactins (for pyridine aba activation).

To delineate their mechanism of action, the effects of the Pyrabactins were examined on mutants in the GA and ABA signaling and biosynthetic pathways. These experiments demonstrate that Pyrabactins require a functional ABA signaling, but not biosynthetic, pathway to inhibit germination suggesting that Pyrabactins activate the ABA signaling pathway. To examine this hypothesis further, whole genome transcript profiling was used and these experiments reveal that ABA and Pyrabactin treatments induce strikingly similar expression profiles. Moreover, to isolate ABA-specific probe sets from germination responsive ones, transcripts that were significantly regulated by multiple germination inhibitors were identified and excluded in our microarray analysis. Collectively our genetic, physiological and transcriptional data suggest that the Pyrabactins inhibit germination by activating the ABA signaling pathway and thus define a new class of synthetic plant growth regulators.

As first steps towards target identification, we have taken two approaches. A natural variation screen has identified several strains hypersensitive to Pyrabactin A and analysis of the Cold Spring Harbor Lab ecotype (CSHL1) has shown that it carries a recessive mutation in a locus designated POD1 (Pyrabactin overdoser) that segregates as a Mendelian trait and maps to the bottom arm of chromosome three; map based cloning of the POD1 locus is ongoing. Additionally an EMS screen for Pyrabactin A resistance has identified several mutations; of current interest is a dominant mutation in PYR1 (Pyrabactin resistant) that maps to the bottom arm of chromosome 4 and is currently being fine mapped.

Our discovery of the Pyrabactins illustrates the successful application of forward chemical genetics in identifying new plant growth regulators. Furthermore, the Pyrabactins should be useful tools for dissection and manipulation of the ABA signaling pathway.

434 Experimental Validation of a Predicted Feedback Loop in the Circadian Gene Network of Arabidopsis

Peter Gould[1], James Locke[2], Andrew Millar[3], Anthony Hall[1]

[1]University of Liverpool, [2]Warwick University, [3]University of Edinburgh

Circadian rhythms, controlled by an endogenous circadian clock, have been conserved through evolution from prokaryotes to eukaryotes. Circadian rhythms not only provide organisms with the ability to prepare for environmental changes caused by sunset and sunrise, but also allow seasonality to be determined, an advantage for flowering in plants and mating and hibernation in animals.

The circadian clock generates rhythms with a period length of ~ 24 hours in a diverse range of processes from developmental to behavioural activities. Although a large percentage of genes in higher eukaryotes show circadian rhythmic expression (5-15% of genes), only a small number of these genes are known to be involved in the circadian clocks central network of interlocked feedback loops.

The central network in Arabidopsis thaliana has been shown to comprise of a feedback loop in which LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), repress the expression of TIMING OF CAB EXPRESSION 1 (TOC1) which is their transcriptional activator. Although this clock mechanism mathematically is able to produce an oscillation it cannot fit with all the experimental data to date. For instance, the model would predict that the cca1; lhy double mutant would be arrhythmic but this is not the case. From experimental data the cca1; lhy double mutant infact has a short period length rhythm that persists over several days.

From mathematical modelling and experimental work we present evidence for the existence of multiple feedback loops in the Arabidopsis central network. Within this network GIGANTEA (GI) has been predicted to act as a component of one of the interlocking loops that is critical for the working of the circadian clock. Through experimental work we have identified a role for GI within this model.

435 The Importance Of RecQ Like Helicases From Plants In DNA Repair And Recombination

Frank Hartung, Stefanie Suer, Holger Puchta
University of Karlsruhe, Faculty of botany II

RecQ helicases are conserved throughout all kingdoms of life regarding both their overall structure and function. The originally described RecQ protein from *E. coli* is a DNA helicase and functions as a suppressor of homologous recombination. Eukaryotic RecQ like proteins are also 3´ to 5´ DNA helicases resolving different recombinogenic DNA structures during replication and DNA repair processes. Recently, even strand annealing activity has been reported for different mammalian RecQ like proteins. The RecQ helicases are key factors in a number of DNA repair and recombination pathways involved in the maintenance of genome integrity. In bacteria only one RecQ protein exists but in eukaryotes the number and structure of RecQ like proteins vary strongly between different organisms. Knockouts of several RecQ like genes cause severe diseases (i.e. WRN, BLM and RTS) in men or harmful cellular phenotypes in yeast. These diseases are often accompanied by genomic instability and cancer predisposition. Despite the fact that plants in general do not develop cancer, the largest number of RecQ like genes per organism has been found in them until now. Arabidopsis and rice possess seven different RecQ like genes each and also the moss *Physcomitrella patens* seem to have a comparable number. Therefore we can anticipate essential and also different roles of the RecQ like proteins in plant DNA metabolism.

A major part of our analyses is focussed on T-DNA knockout mutants of several RecQ like genes in *Arabidopsis thaliana,* investigating their behavior in DNA repair and recombination pathways. We found miscellaneous phenotypes of single and double mutants regarding the homologous recombination (HR) frequency using ß-glucuronidase containing HR reporter lines. Furthermore, we also analysed the genes of different RecQ interacting proteins in respect to their role in HR.

436 GATEWAY vectors for plant genome analysis

Mansour Karimi, Bjorn De Meyer, Rudy Vanderhaegen, Christiane Genetello, Dirk Inze, Pierre Hilson
Department of Plant System Biology (PSB), Vlaams Interuniversitair Instituut voor Biotechnologie, Universiteit Gent, Technologiepark 927, B-9052 Gent, Belgium

The Gateway™ technology (Invitrogen) has been developed to facilitate the transfer of DNA segments into and between plasmids by recombinational cloning. We have constructed a large collection of Gateway-compatible destination vectors for a wide range of gene function analyses in transgenic plant cells. Taking advantage of the novel Multisite recombination Gateway cassettes, plant binary destination vectors have also been created in which two or three segments can be transferred from source entry clones and pasted contiguously in a single LR clonase *in vitro* reaction. We are currently creating a collection of universal pEntry clones in diverse Multisite pDonor vectors to expand the range of plant expression constructs that can be produced straightforwardly using the Multisite Gateway system. Furthermore, vectors for the analysis of monocotyledons genome have been created. All our destination vectors carry one of three plant selectable markers coding for resistance to kanamycin (*nptII*), hygromycin (*hpt*) or glufosinate ammonium (*bar*). All recombinational cloning cassettes are also available in high copy number plasmids. This collection of vectors is a useful resource for the plant research community and can be obtained on line (http://www.psb.ugent.be/gateway). The web site also includes other accessions and provides recombinational cloning instructions, as well as experimentally verified sequences, maps and Vector NTI files for each plasmid.

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437 High-throughput platform for expression in Arabidopsis protoplasts and cultured cells

Le Son Long Nguyen, Rudy Vanderhaeghen, Mansour Karimi, Alain Goossens, Pierre Hilson

Department of Plant Systems Biology (PSB), Flanders Interuniversity Institute for Biotechnology (VIB), Ghent University, Technologiepark 927, B-9052 Ghent, Belgium

Sequence information and genome annotation are improving at an impressive pace but functional ontology is still inexistent or rudimentary for most genes. Even though they are not suited for complex organismal studies, assays in plant protoplasts and cultured cells have proven very useful to dissect a broad range of molecular mechanisms including signaling and metabolic pathways as well as transcriptional regulatory networks. We are building the framework enabling large-scale systematic screens in such cells. (1) Protocols for transfection of tobacco and Arabidopsis protoplasts have been adapted for automated handling in multi-well plates. Our current experimental set-up allows processing of up to 200 samples per day. (2) Vectors were constructed compatible with efficient Gateway recombinational cloning (www.psb.ugent.be/gateway) and designed for gene overexpression, promoter studies or silencing via RNA interference. They include reporter plasmids for standardized assays based on the dual firefly/*Renilla* luciferase enzymatic system (3) We are currently developing the hardware and software necessary to record and analyze cell growth, *in vivo* reporter gene expression and other cellular features at the microscopic level.

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438 HY5 Interacting Proteins Isolated by Genome Wide Screen of Protein-Protein Interaction of Plant Transcription Factors

Hye Jin Kim, Ju Hwoan Kang, Young Hun Song, Su Young Shin, Na Young Song, Geon Hui Son, Jong Hong

Department of Biochemistry and Environmental Biotechnology Research Center, Division of Applied Life Science, Gyeongsang National University, Jinju, 660-701, Korea

The comprehensive analysis of transcription factor interaction can provide fundamentally important information for understanding the complex regulation of gene expression in plant growth and development. The construction of transcription factor (TF) ORFeome library in a yeast two hybrid vectors can provide a valuable tool in isolating novel interacting TFs to the protein of interest. We have constructed Arabdisopsis TF ORFeome library which contains 1400 TFs in a correct reading frame to the prey vector (pDEST32 Gal4AD) using gateway recombination cloning system (Invitrogen).

The Arabidopsis transcription factor HY5 is one of the best characterized bZIP transcription factor that act as positive regulator of photomorphogenesis. The HY5 is also involved in other developmental process such as root development, hormone responses, and flowering. This indicates that HY5 regulate many genes by interacting with multiple regulatory factors. Using the full length HY5 protein as a bait we have isolated 49 different transcription factors which include 8 B-box zinc finger factor, 9 bZIP factors, 6 C2H2, 5 AP2/EREBP, 2 MYB , 4 TCP proteins, and others. Thirty TFs also interacted with HYH, a HY5 homolog, an indication that the interaction identified by high throughput analysis is specific. In addition, more than 20 HY5 interacting proteins are shown to interact with COP1, a negative regulator that specifically targets HY5 for degradation via 26S proteosome in the dark through direct physical interaction. This study shows that the Arabidopsis TF ORFeom library is a powerful tool to identify many novel interacting factors of biological interest (Supported by BK21 program and CFGC of 21C Frontier Research Program, KOSEF)

439 A Comparison of Full Versus Partial ^{15}N Incorporation for Metabolic Labeling in Arabidopsis Thaliana

Edward Huttlin[2, 1], Adrian Hegeman[2, 1], Amy Harms[1], Michael Sussman[2, 1]

[1]University of Wisconsin Biotechnology Center, [2]University of Wisconsin Biochemistry Department

Over the past several years a variety of isotope-assisted quantitative proteomics techniques have been developed, allowing use of tandem mass spectrometry for the simultaneous comparison of the identities and abundances of hundreds or thousands of proteins within pairs of biological samples. These approaches include in vitro labeling techniques such as ICAT and ^{18}O labeling which introduce an isotopically-labeled tag onto each sample during sample preparation, as well as in vivo metabolic labeling techniques in which an isotopic label is incorporated into the organism from its media during normal growth and development. For all of these approaches, after incorporation of either a light or a heavy isotopic tag, the control and experimental samples are combined and processed together. Because the tagged peptides are essentially chemically identical, they provide an excellent internal control for all subsequent steps in sample preparation and analysis. Yet using mass spectrometry we can differentiate among peptides from each sample due to the mass difference between the heavy and light isotopic tags. Because metabolic labeling allows combination of control and experimental samples through all steps of sample processing including tissue homogenization and protein extraction, it provides perhaps the ideal internal control.

While metabolic labeling provides an elegant control for all steps in sample preparation, presently some technical challenges limit its widespread application, especially in intact organisms such as plants. First, achieving full incorporation of an isotopic label such as ^{15}N into plants and other higher organisms is challenging and may require unnatural growth conditions that limit the biological questions to which it may be applied. Additionally, when applied in a high throughput manner these approaches require informatics tools for automated data analysis. We have compared traditional ubiquitous ^{15}N metabolic labeling in Arabidopsis with a new approach using partial incorporation of ^{15}N (based on Whitelegge et al. Phytochemistry 65 (2004) 1507-1515) to produce changes in the isotopic envelopes of each peptide that can be used for quantitative comparison. While both approaches require significant informatics tools for analysis, the latter is perhaps more amenable for labeling under a wider variety of conditions. We will present a comparison of traditional metabolic labeling with partial metabolic labeling in Arabidopsis whole tissue with respect to numbers of peptide and protein identifications as well as quantitative accuracy and dynamic range. We will also discuss our current strategies for automated data analysis using both approaches.

440 Understanding the TGA transcriptional network using ChIP-chip and expression arrays

Francoise Thibaud-Nissen[1], Christopher Johnson[2], Hank Wu[1], Julia Redman[1], Yuelin Zhang[3], Xin Li[3], Jonathan Arias[2], Christopher Town[1]

[1]The Institute for Genomic Research, Rockville MD, USA., [2]University of Maryland, Baltimore County, MD, USA., [3]University of British Columbia, Vancouver BC, Canada

In order to understand the transcriptional network of TGA factors in Arabidopsis and its role in systemic acquired resistance, we are conducting ChIP-chip studies using TGA antibodies and analysis of SA-induced changes in expression in wild-type and *tga* mutants. The TGA transcription factors TGA2, TGA5 and TGA6 have been found to play a redundant and essential role in the onset of systemic acquired resistance. In a preliminary analysis we have identified over 50 putative binding sites for TGA2 through a ChIP-chip approach using Arabidopsis whole-genome arrays, 55% of which contain a single palindromic octamer TGACGTCA. Additional ChIP-chip data using TGA5 show substantial overlap in the binding sites of TGA2 and TGA5. In parallel, the effect of the SA treatment on gene expression in wild-type plants and *tga2tga5*, *tga6* and *tga2tga5tga6* mutants was determined using Affymetrix ATH1 arrays. We observed substantial and very similar changes in expression upon SA application in the wild-type, the single and the double mutant. In particular, genes coding for kinases and proteins involved in the response to biotic stimuli are overrepresented among the genes differentially regulated by SA. However, SA induced very few changes in gene expression in the triple mutant. Hierarchical clustering of experimental samples based on gene expression revealed that the 18-hour mock-treated samples of the triple mutant cluster with SA-treated samples of the wild-type, single and double mutants, indicative of high basal levels of genes usually induced by SA in the untreated *tga2tga5tga6* plants.

Research supported by the NSF 2010 Program