1 Global Analysis of Genomes and Proteomes: Lessons from Yeast and Humans

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The genomes of a wide variety of organisms have now been sequenced; a major challenge ahead is to identify genes and their encoded projects and to understand their function and regulation. For the case of budding yeast a wide variety of approaches have been undertaken to identify genes and proteins and characterize gene and protein function on a global scale. The analysis of gene and protein function using transposon tagging, large scale gene inactivation, protein-protein interactions, and biochemical assays (e..g protein micorarrays) have been carried out on a global scale. Using bioinformatic methods, the integration of multiple approaches and even multiple data sets using the same approach greatly enhances the information and accuracy of the results. The analysis of gene regulation using expression analysis and identification of binding sites of transcription factors is beginning to reveal complex regulatory networks. These networks are even more informative when integrated with other types of information such as protein-protein interactions and protein complex formation. I will present the current state of different global projects involved in identifying genes and analyzing their function in yeast. Extension of similar projects to humans will also be discussed. Emphasis will be given on 1) several projects from our laboratory (gene identification, proteome arrays and global analysis of gene regulation) and 2) unexpected findings 3) how this information is utilized by the scientific community.

2 Functions of the *Arabidopsis* microRNA and siRNA Systems

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Plants and other multicellular eukaryotes have several small RNA pathways for developmental control, defense against viruses and mobile genetic elements, and modification of chromatin. These depend on microRNAs (miRNAs), which arise from processing of structured fold-back precursors, and short interfering RNAs (siRNAs), which originate from both strands of long dsRNA molecules. Genetic analysis of the DICER-LIKE family and other genes involved in small RNA formation revealed at least three distinct small RNA generating systems in Arabidopsis, one for miRNAs and at least two for siRNA formation from endogenous and invasive elements. miRNAs function in RISC or RISC-like complexes as negative regulators of target mRNA expression. In Arabidopsis, several dozen target mRNAs that belong to three groups were identified. These groups include mRNAs coding for 1) transcription factors that govern meristem identity and organ polarity, 2) miRNA metabolic factors, such as DICER-LIKE1 (DCL1), and 3) proteins of unknown function. All mRNA targets that have been validated to date are sensitive to miRNA-guided cleavage by a mechanism similar (or identical) to the RISC-mediated cleavage mechanism that occurs during RNA silencing. Interestingly, several plant viruses that encode RNA silencing suppressors inhibit miRNA-guided cleavage of target mRNAs, resulting in infected plants that display a variety of developmental defects.

3 Genetics of gene silencing in Arabidopsis

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Our laboratory studies epigenetic mutations at the SUPERMAN and FWA genes, which are caused by heritable changes in DNA methylation and chromatin structure of these loci. We have identified the DRM2 de novo DNA methyltransferase as a critical component of a system that establishes methylation and silencing at both SUPERMAN and FWA. Furthermore, we have shown that three factors are required for the maintenance of DNA methylation and silencing at SUPERMAN. The first is the DNA methyltransferase CHROMOMETHYLASE3, which appears to be the major enzyme controlling CpNpG methylation in the genome. The second is KRYPTONITE, a lysine 9 histone H3 protein methyltransferase gene, which is also required for the maintenance of CpNpG methylation. The third is ARGONAUTE4, a protein required for the accumulation of 25 nucleotide siRNAs that correspond to methylated and silenced loci. Current data concerning the mechanism of action of these proteins in gene silencing will be presented.

4 MicroRNAs and plant development

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MicroRNAs (miRNAs) comprise a large class of endogenous ~22-nt RNAs, some of which are known to direct posttranscriptional gene regulatory machinery to mRNA targets. MicroRNAs are present in plants as well as animals, indicating that this class of noncoding RNAs arose early in eukaryotic evolution. As with the animal miRNAs, the plant miRNAs are processed from evolutionarily conserved hairpin precursor molecules through the action of Dicer, and a single miRNA ultimately accumulates from one arm of each precursor molecule. Plant miRNAs differ from animal miRNAs in that their hairpin precursors are more variable in size. Plant miRNAs also are more likely to pair to mRNAs with nearly perfect complementarity, allowing targets for plant miRNAs to be readily and convincingly predicted.

A systematic computational search for targets shows that plant miRNAs have a remarkable propensity to target messages of transcription factors, particularly members of transcription factor families involved in developmental patterning or cell differentiation. Several of the predicted targets that are not transcription factors have links to RNA metabolism. These include *DCL1* and *AGO1*, plant Dicer and Argonaute family members implicated in miRNA processing and function, which suggests a negative feedback mechanism controlling their expression. The targeting of developmental transcription factors suggests that many plant miRNAs function during cellular differentiation to clear key regulatory transcripts from daughter cell lineages. Genetic, molecular, and transgenic data are consistent with this hypothesis.

5 Genetic analysis of RNA-directed DNA methylation in *Arabidopsis*

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Recent work has shown that epigenetic alterations, such as DNA cytosine methylation and histone modifications, can be targeted to specific regions of the genome by short RNAs derived from cleavage of double stranded RNA (dsRNA). The first RNA guided epigenetic modification to be discovered was RNA-directed DNA methylation (RdDM). RdDM is a highly specific process that was detected almost ten years ago in viroid-infected transgenic plants (1). Methylation resulting from RdDM is confined largely to the region of RNA-DNA sequence identity, suggesting that RNA-DNA base pairing is the signal for *de novo* methylation. RdDM leads to dense methylation at most cytosines, not just those in symmetrical C(N)G nucleotide groups. To understand the relationships among RNA signals, DNA methylation and chromatin modifications, we are carrying out a genetic analysis of an RNA-mediated transcriptional gene silencing (RTS) system in *Arabidopsis* (2). The analysis has recovered several mutants falling into three complementation groups. One mutant, *rts1*, is defective in a putative histone deacetylase, HDA6, that is needed to enhance C(N)G methylation induced by dsRNA (3). Two other mutants, *rts2* and *rts3*, have been identified and are currently being mapped and characterized. *Rts3* maps to a region of the genome that so far contains no known epigenetic mutation and thus likely represents a novel component of a transcriptional gene silencing pathway. The most recent results from the mutant screen will be presented. Our work is funded by grants Z21-MED and P15611 from the Austrian FWF.

1. Wassenegger et al.(1994) Cell 76: 567-576.
2. Aufsatz et al.(2002) Proc. Natl. Acad. Sci. USA 99, 16499-16506.
3. Aufsatz et al.(2002) EMBO J. 21: 6832-6841.

6 Regulation and Functions of TCH4 and related XTHs

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Xyloglucan endotransglucosylase/hydrolases (XTHs) are enzymes capable of modifying a major component of the plant cell wall, yet the physiological functions of these enzymes remain unclear. *TCH4*, encoding XTH22, is unusual in that it is up regulated in expression in response to a variety of stimuli including mechanical stimuli, such as touch and wind, temperature extremes, darkness and some hormones. Expression induction by these diverse stimuli may occur through a common *cis* regulatory region. We have conducted screens for mutants altered in *TCH4 XTH* expression using luminescence emission of *TCH4::LUC* transgenics as an assay. These *TCH4* expression (*tex*) mutants will lead to insight into the mechanisms and machinery involved in perception and response to stimuli that regulate the Arabidopsis *TCH* genes. We are also investigating the functions and expression patterns of the Arabidopsis *XTH* family to gain insight into the physiological consequences of xyloglucan modifications in the cell wall.

7 Functional dissection of intercellular protein trafficking in *Arabidopsis*

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The intercellular trafficking of regulatory proteins and mRNAs provides a novel cell-to-cell communication mechanism in plant development. The maize *KNOTTED1 (KN1)* homeobox gene acts non-cell autonomously in the maize leaf, and KN1 was the first plant protein shown to traffic cell-to-cell, presumably through plasmodesmata. We have compared the intercellular trafficking of green fluorescent protein (GFP) fusions of KN1 and *Arabidopsis* KN1-related (KNOX) proteins SHOOTMERISTEMLESS (STM) and BREVIPEDICILLUS (BP) to that of the viral movement protein (MP) from turnip vein clearing tobamovirus. We found tissue-specific regulation of GFP~KNOX protein trafficking during development. For example, GFP~KN1 can traffic from the inner layers of the leaf to the epidermis, but not in the opposite direction from epidermis to mesophyll. In contrast, the GFP~MP fusion trafficked readily from epidermis to mesophyll. GFP~KNOX fusions were however able to traffic out of the epidermal (L1) layer in the shoot apical meristem, indicating that movement out of the L1 is developmentally regulated. Linking this phenomenon to a functional significance of intercellular trafficking, we found that the L1 layer specific expression of KNOX proteins was able to partially complement the strong *stm-11* mutant allele. However, a cell autonomous KNOX protein fusion failed to complement when expressed only in the L1. These results suggest that the activity of KNOX proteins is maintained following intercellular trafficking, and that trafficking may be required for their normal developmental function.

To gain further insight into the mechanism of intercellular protein movement, we have designed genetic screens using GFP and other reporters. In particular, a screen for mutants affected in intercellular GFP diffusion identified a number of candidate loci that may regulate plasmodesmal size exclusion limit. We also describe a novel screen for trafficking signals and mutants using trichome rescue by KNOX fusion reporters.

8 *fk*, *hyd1* and *smt1/cph* mutants and sterol biosynthesis inhibitor-treated seedlings exhibit incomplete cell walls and reduced levels of cellulose

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A critical role for sterols in plant growth and development is underscored by the characterization of three Arabidopsis sterol biosynthesis mutants: *fackel*(*fk*), *hydra1* (*hyd1*) and *sterol methyltransferase 1/cephalopod* (*smt1/cph*). We are using both sterol profiling and ultrastructural analysis to investigate the primary defects underlying the mutant phenotypes. Comprehensive GC-MS analysis of *hyd1* in comparison to *fk* reveals an abnormal accumulation of unique sterol intermediates in each case. Despite distinct differences in sterol profiles, *fk*, *hyd1* as well as *smt1/cph* share ultrastructural features such as incomplete cell walls and aberrant cell wall thickenings in embryonic and post-embryonic tissues. The common defects are coupled with ectopic callose and lignin deposits, and reduced levels of cellulose that cannot be attributed to altered sugar profiles. Sterol biosynthesis inhibitors 15-azasterol and fenpropimorph also cause cell wall gaps in dividing roots and a reduction in bulk cellulose, corroborating that the cell wall abnormalities are due to altered sterol composition. Our results demonstrate that sterols are crucial for cellulose synthesis in the building of the plant cell wall.

9 GPI-anchored proteins are required for morphogenesis, but not cell division

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Four different *PEANUT* genes were identified in a genetic screen for embryo mutants phenotypically similar to null mutations in the cellulose synthase gene *RSW1*. In addition to radial swelling, *peanut* mutants show a delayed transition from the globular to heart stage of morphogenesis, as well as variable cotyledon number. Although *peanut* mutants are seedling lethal, *peanut1* cells are able to proliferate as callus. *peanut1* mutants display alterations in cell wall ultrastructure, and chemical analysis of *peanut1* cell walls revealed decreased crystalline cellulose, as well as increased levels of pectin and of the hemi-cellulosic neutral sugars. We cloned the *PEANUT1* gene based on its map position. The predicted PNT1 protein has significant similarity to mammalian PIG-M, an ER-localized mannosyltransferase that is required for production of the core glycosylphosphatidylinositol (GPI) anchor. GPI anchors are added to proteins destined for extracellular secretion, and serve to attach these proteins to the plasma membrane without the protein itself being integrated into the membrane.

Synthesis of the GPI anchor is a processive process, and thus mutations that interfere with GPI anchor biosynthesis would be expected to result in a lack of the complete anchor. In mammalian cells, mutations that interfere with the transfer of a functional GPI anchor to the corresponding protein result in the degradation of said protein. To test whether *peanut* mutations lead to degradation of GPI-anchored proteins, we looked at the stability of three GPI-anchored cell wall-related proteins in *peanut* mutants. SKU5, a cell wall localized protein of unknown function, was undetectable in extracts of all four peanut mutants. In addition, levels of PMR6, a putative pectate lyase, and COBRA, a protein proposed to regulated cellulose deposition, were also drastically reduced in *peanut1* callus extracts. Our results suggest that *peanut* mutants have a general defect in GPI-anchored proteins, and underscore the importance of these proteins in cell wall biogenesis and morphogenesis. Interestingly, while GPI anchored proteins are required for organized multicellular growth, they are apparently dispensable for the disorganized cell proliferation typical of callus tissue.

10 Two distinct functions of callose

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Exine, the outer layer of the pollen wall, is pollen specific and comprised of a polymer known as sporopollenin. Exine is formed before pollen is released from the anther, and formation of its distinct pattern is thought to require intracellular patterning signals from the developing microspores. We developed an adhesion assays to quantify the first step of pollination, pollen-stigma binding, and showed that exine mediates the species-specific interactions. Using a rapid stigma-binding assay, we screened for mutants defective in pollen adhesion. This screen yielded *lap1*, a mutation that disrupted pollen wall development and exine pattern formation. We mapped the *lap1* mutation to a gene that is predicted to encode a putative callose synthase expressed in flower buds. The principal features of exine patterns are established soon after meiosis while newly formed meiocytes are surrounded by callose. We found that all *lap1* mutants have a defect in callose deposition in the walls that surround the pollen mother cells, indicating that the callose layer engulfing meiocytes is essential for establishment of the exine layer pattern.

Pollen tube walls of flowering plants contain callose that resides in the tube wall and forms the plugs that separate the growing tip from the evacuated tail. The callose plug keeps the pollen cytosol within a reasonably small volume and therefore has been thought to play an important role for fertilization. We also found that *lap1-1* pollen tubes lack callose plugs and are also deficient in callose accumulation within pollen tube walls. Surprisingly, *lap1* tubes are fertile, indicating that callose is not essential for pollen tube fertility.

11 Distorted Trichome Mutants and the Actin Cytoskeleton

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Most non-dividing plant cells display asymmetry: regulated organelle positioning, transport activity, and cell surface properties are examples. Cell asymmetry is fundamentally important for the specialized functions of cells, tissues, and organs. All cells use a filamentous network of cytoskeleton and associated proteins to generate and maintain a polarized cytoplasm. Despite many years of research, the signaling pathways that control cytoskeletal organization and the function of particular cytoskeletal arrays are not understood mechanistically. We are using the single cell developmental process of trichome development and molecular genetics as an entree into actin-based cell shape control. Arabidopsis trichomes or leaf hairs are unicellular, highly polarized epidermal cells, which display distinct requirements for both the microtubule and actin-filament cytoskeletons. One long-term goal of our research is to use the "distorted group" mutants to better understand actin organization and function in plant cells. The "distorted group" of trichome mutants displays cell shape defects that precisely coincide with the developmental transition to actin-dependent cell growth. We and others identified at least eight loci that, when mutated, give rise to a distorted trichome phenotype. Recent experiments demonstrate that some of the *DISTORTED* genes encode components of the ARP2/3 complex. For example allele sequencing and western blot experiments clearly demonstrate that a defects in the *AtARP3* gene cause the *distorted1* (*dis1*) phenotype. *dis1* plants not only display altered sensitivity to the actin-depolymerizing drug latrunculin B, but also possess less organized cytoplasmic actin bundles in the branches of developing trichomes compared to the wild type. ARP3 is one of seven subunits that assemble into a complex known as the ARP2/3 complex. In animal cells the ARP2/3 complex is an important nucleator of actin filaments that drives both membrane protrusion and organelle motility. This talk will summarize our experiments that use the "distorted group" mutants to understand the functions of the ARP2/3 complex in plant cells.

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12 Dissection of the autophagic process in *Arabidosis thaliana* involving the APG protein group

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Autophagy is an important route for the bulk removal of cytosolic proteins and organelles in all eukaryotes especially in cells/tissues undergoing apoptosis, or subjected to stress and starvation. Here, portions of the cytoplasm are engulfed in autophagic vesicles, which are then transported to the lysosome/vacuole for degradation by resident proteases. Although important during seed germination, growth during nutrient limiting conditions and senescence, the molecular mechanisms underpinning autophagy in plants are not well understood. Recently, a Tor kinase-activated protein modification system involving the APG8 and APG12 polypeptide tags has been shown to be essential for autophagy in yeast. Using the yeast genes as queries, we have identified an orthologous pathway in the plant *Arabidopsis thaliana*. In addition to genes encoding APG8 and APG12, proteins required for their activation, including the APG1 kinase, and ultimate ligation to target proteins and/or phospholipids have been identified. Nine distinct genes encoding APG8 are evident indicating that the *Arabidopsis* pathway may be more functionally diverse. Using a reverse genetic approach, we have generated a library of knockouts in many of the *APG* loci. Phenotypic analyses of disruptions in *APG7* and *APG5*, genes encoding the enzyme responsible for APG8/12 activation and the target of APG12 conjugation, respectively, indicate that the pathway is not essential for normal growth and development in *Arabidopsis*. However, the mutants are hypersensitive to N- and C-limiting media and undergo premature senescence, suggesting that the pathway has a role during nutrient-limiting conditions and extensive nutrient remobilization. We have also developed fluorescently tagged APG pathway proteins to aid in tracking the formation of autophagic vesicles and their subsequent delivery to the vacuole *in planta*. In addition, molecular markers for various vesicles and intracellular compartments will be used to observe vesicle formation. We anticipate that these studies will reveal the importance of autophagy in protein recycling and cellular maintenance in plants.

13 The Rab GTPase, AtRabA4b, Localizes to the Tips of Growing Root Hairs

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Spatial and temporal control of cell wall deposition plays a unique and critical role during growth and development in plants. Yet little is known about the molecular mechanisms by which cell wall polysaccharides are synthesized and secreted, or how polarized deposition occurs in differentiating plant cells. We have focused on Rab GTPases, a class of regulatory molecules that control membrane trafficking in eukaryotes, as a mechanism to identify endomembrane compartments that are involved in cell wall biogenesis in *A. thaliana*. To this end, we have examined the function of a plant Rab GTPase, AtRabA4b, during polarized expansion in developing root hair cells. When AtRabA4b is fused with yellow fluorescent protein, the resulting EYFP-AtRabA4b specifically localizes to the tips of growing root hair cells in *A. thaliana*. Depolymerization of actin microfilaments with latrunculin B causes inhibition of root hair growth, and correlates with loss of EYFP-AtRabA4b tip-localization. Localization of EYFP-AtRabA4b in root hair development mutants is also consistent with a role in proper tip-growth of root hairs. Using cell fractionation methods we have identified compartments labeled with EYFP-AtRabA4b that co-localize with trans-Golgi markers. We therefore hypothesize that AtRabA4b, which shares a high degree of similarity to yeast Ypt31/32 and mammalian Rab11, is localized to a post-Golgi compartment that is involved in the polarized deposition of new cell wall during tip-growth in root hair cells. Further characterization of the AtRabA4b-labeled compartment and potential cargo molecules will be presented.

14 Maintaining an edge: telomere structure and function in *Arabidopsi*

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We are exploiting *Arabidopsis* as a model for telomere biology in higher eukaryotes. One important goal in the field is to understand how cells distinguish natural, fully capped chromosome ends from ends that lack a functional telomere. We previously showed that *Arabidopsis* mutants lacking telomerase are viable for up to ten generations, but progressive telomere erosion leads to the onset of growth and developmental defects by the sixth generation. Later generations are characterized by massive end-to-end chromosome fusions. When a telomerase deficiency is combined with a deficiency in Ku70, a protein essential for double strand break repair via NHEJ, telomere erosion is dramatically accelerated with double mutants reaching the terminal phenotype by the third or fourth generation. Remarkably, the incidence of anaphase bridges is the same in *tert* mutants as in *tert ku70* mutants, arguing that NHEJ may not be involved in fusing dysfunctional telomeres.

We now describe a novel PCR approach to follow telomere dynamics in telomerase-deficient plants. We show that the shortest functional telomere is approximately 200-300 bp. The range of telomere length between the shortest and the longest telomere in the population decreases in successive generations, suggesting that either the rate of telomere shortening decreases for shorter telomeres or that the shortest telomeres cannot be detected in the assay because they are engaged in end-to-end fusions. We also used PCR to examine approximately 70 different chromosome fusion junctions in *tert* and *tert ku70* mutants. Most fusions consisted of heterologous chromosome arms; sister fusions were rarely detected. The majority of junctions involved apposition of one truncated subtelomeric sequence with the telomere tract of another chromosome. Intriguingly, in 40% of the fusion junctions in *tert* mutants harbored small insertions, typical of NHEJ. In contrast, only 7% of the *tert ku70* fusions had inserted DNA. Further, we observed significantly more telomere-telomere fusions in the double mutant. Together, these data suggest that Ku70 may play a role in fusing dysfunctional telomeres, but in the absence of Ku function alternative means are used to join uncapped chromosome ends.

15 Activation of telomerase by synergistic action of a novel zinc finger protein and auxin

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Telomerase is a specialized reverse transcriptase responsible for synthesizing and maintaining telomeric DNA. In plants, telomerase expression is restricted to reproductive organs, embryos, and rapidly dividing cells such as immortalized, dedifferentiated cells growing in culture. There is a strong correlation between cell cycle progression and telomerase expression in all organisms. In synchronized tobacco cells, telomerase activity is restricted to late S-phase. Exposure to IAA induces telomerase activity to higher levels and an earlier appearance during S phase. To identify genes that regulate telomerase in Arabidopsis, we assayed 7,976 activation tagged lines for ectopic telomerase activity in their leaves. We designated the first of four mutants we recovered *telomerase activator 1-1D*. Expression of telomerase in the leaves was dominant and genetically linked to the activating T-DNA. We recovered the activating T-DNA and flanking plant DNA from *tac1-1D* and identified the activated gene in this line as one encoding a novel protein with a single zinc-finger motif. This *TELOMERASE ACTIVATOR1* gene lies 78 kilobases away from the activating T-DNA, indicating that the CaMV 35S enhancers in these lines can act over very long distances. The cell cycle was not perturbed in the mutant, the first time in any organism that expression of telomerase has been separated from progression through the cell cycle. Surprisingly, callus from *tac1-1d* plants grows in the absence of exogenous auxin, confirming the previously reported link between this hormone and telomerase. *yucca* mutants, with increased levels of IAA, do not express telomerase ectopically, indicating that higher concentrations of auxin alone are not sufficient to induce the enzyme. However, lowering auxin concentrations in the *tac1-1d* background by expressing a bacterial enzyme that conjugates free IAA to lysine, prevents TAC1-mediated telomerase expression. These results, along with other phenotypes of the mutant, suggest that TAC1 potentiates some, but not all, responses to endogenous auxin, including activation of telomerase. Characterization of our additional telomerase activation mutants should help to further elucidate pathways that controls expression of this essential enzyme.

16 Identification of key DNA elements critical for centromere function

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The centromere is involved in several critical functions that culminate in the proper partitioning of replicated chromosomes during mitosis and meiosis. Chromosome missegregation leads to aneuploidy and abnormal karyotypes, which in humans, can cause cancer, decreased fertility, spontaneous abortions and trisomy syndromes such as Downs and Klinefelters. Arabidopsis is the only higher plant with mapped centromeres and extensive centromere sequence, making it an excellent system to analyze centromere function and the causes and effects of missegregation events. Moreover, with this information in hand, it becomes possible to clone centromere DNA elements and to construct cell autonomous plant mini-chromosomes. Towards this end we have developed novel assays to identify DNA segments that confer centromere activity. These assays include identification of DNA fragments that can form a second, dicentric centromere on a wildtype chromosome and sequences that confer proper segregation to a cell autonomous mini-chromosome. Using these assays we have identified key centromere DNA signatures that are necessary for function. Furthermore, we have developed a robust, innovative system to monitor chromosome segregation during meiosis. Using this system we have determined the fidelity of chromosome segregation during meiosis and monitored the effects of different agents that increase the incidence of chromosome loss.

17 Adaptive evolution of the centromere-specific histone gene in Brassicaceae

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Centromeric DNA, being highly repetitive, has been refractory to evolutionary analysis. However, centromeric structural proteins are encoded by single-copy genes, and these can be analyzed using standard phylogenetic tools. The centromere-specific histone, CenH3, replaces histone H3 in centromeric nucleosomes and is required for the proper distribution of chromosomes during cell division. Whereas histone H3s are nearly identical between species, CenH3s are divergent, with an N-terminal tail that is highly variable in length and sequence. We find that in the Brassicaceae, as in Drosophila, the N-terminal tail of CenH3 is rapidly and adaptively evolving, as revealed by comparison of replacement and synonymous changes in coding sequences. In addition, the Loop 1 region, previously shown to be necessary and sufficient for CenH3 localization in Drosophila, is also evolving rapidly. These findings are consistent with the hypothesis that CenH3-containing nucleosomes act as adapters in both plants and animals to facilitate the interaction between the rapidly evolving satellite DNA and the conserved kinetochore machinery.

18 Regulating Meiotic Recombination in Arabidopsis thaliana

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Crossover interference and the interaction between the recombination machinery and chromatin are two of the most potent mechanisms that govern the genome-wide distribution of crossovers during meiosis. To better understand these processes we are exploring the regulation of recombination events in Arabidopsis taking advantage of the quartet mutation, which enables the use of tetrad analysis. We have demonstrated that inhibitors of DNA methylation or histone de-acetylation have profound effects on the levels of recombination specifically in heterochromatic regions of plant chromosomes without modulating overall recombination levels. This pattern indicates a role for spatial regulation distinct from overall activity. To facilitate future studies of recombination mechanisms, we have developed a rapid and reliable visual screen for recombination events. In this screen the post-meiotic expression of GFP and MUG-based (8-hydroxyquinoline-beta-D-glucuronide) markers are used to assay the segregation of chromosome segments in pollen tetrads. This assay system makes it feasible to visually assess recombination frequencies, detect subtle mutant phenotypes and utilize large populations in a high-throughput manner to detect rare events. Previously we reported using tetrad analysis to show that crossovers on chromosomes 1, 3 and 5 display distributions consistent with a mixture of both interference sensitive and interference insensitive recombination events. That analysis was not sensitive enough to draw conclusions for chromosomes 2 and 4. Interestingly, both chromosomes 2 and 4 harbor large nucleolar organizing regions which have been proposed to function as cis-acting pairing centers in other species. Current efforts are focused on expanding our analysis of chromosomes 2 and 4 to explore the possibility that the NOR bearing chromosomes of Arabidopsis may experience a distinctly different interference pattern than the rest of the genome. Progress on these efforts will be reported.

19 Understanding flavonoid metabolism in three dimensions

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Flavonoids, a major class of plant secondary metabolites, appear to be synthesized on a multienzyme complex or metabolon, as first proposed by Helen Stafford in 1974. This organization has important implications for controlling the activity, efficiency, and specificity of cellular metabolism and, in the case of flavonoids, may offer a critical mechanism for mediating the synthesis and intracellular deposition of diverse endproducts. In the past several years we have published evidence for specific interactions among flavonoid enzymes and for colocalization of these proteins in *Arabidopsis* roots. We are now working to develop a three dimensional model for flavonoid metabolism based on authentic crystal structures and homology models of the individual enzymes. This endeavor is based both on computational approaches to model protein interactions and on the use of small angle neutron scattering to probe the shapes of multienzyme structures in solution. Our ultimate goal is to establish the molecular basis of enzyme complex assembly and regulation, an essential consideration for metabolic engineering in plants and other organisms.

20 Manipulation of NifS-like Proteins ? Effects on Selenium and Sulfur Metabolism

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NifS-like proteins catalyze the formation of elemental S and alanine from cysteine or of elemental Se and alanine from selenocysteine. Cysteine desulfurase activity is required to produce the S of Fe-S clusters, while selenocysteine lyase activity is needed in many organisms for the specific incorporation of Se into selenoproteins. Excess Se is toxic to cells due to non-specific incorporation of Se-amino acids into proteins. In plants the chloroplast is the location of (seleno)cysteine formation, as well as a location of Fe-S cluster formation. The goal of these studies is to study the involvement and importance of NifS-like proteins for chloroplast selenium and sulfur metabolism. In one approach a mouse selenocysteine lyase (SL) was expressed in the chloroplast or cytosol of *Arabidopsis thaliana*. The SL transgenics showed normal phenotypes on soil or MS-agar medium. Interestingly, cytosolic SL plants show a marked increase in tolerance to selenium, while chloroplastic SL lines have reduced Se tolerance as compared to wildtype plants. Reduced (non-specific) Se incorporation in proteins was found for both cytSL and cpSL lines, which may explain the enhanced tolerance of the cytSL plants. The Se hypersensitivity of the cpSL lines may be explained by interference of produced elemental Se with chloroplastic Fe-S cluster formation. During these studies an *A. thaliana* homologue of the mouse SeCys lyase (AtCpNifS) was discovered and characterized. AtCpNifS is located in the plastids and expressed in all tissues. It has both SL and Cys desulfurase activity, with a 300-fold higher activity towards the Se substrate. Antisense AtCpNifS transgenics show hypersensitivity to Se, indicating a role for AtCpNifS in Se tolerance. In addition, AtCpNifS may play a role in plastidic Fe-S cluster formation, as suggested by the presence of Arabidopsis homologues of bacterial NifU and NifA genes. Their gene products are predicted to be plastidic, and may interact with AtCpNifS to form Fe-S clusters, in analogy to bacterial metabolism. The one NifA and four NifU homologues were cloned from Arabidopsis, and their interaction with AtCpNifS is presently being investigated.

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21 AGPase is activated by post-translational redox-modification in leaves

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ADPglucose pyrophosphorylase (AGPase) catalyses the first committed reaction in starch synthesis in plants. It has recently been shown in potato tubers that this enzyme is subject to redox-regulation (Tiessen et al., 2002), involving the formation of an intermolecular cysteine brigde between the two AGPB subunits of the heterotetrameric holoenzyme. Here we show that AGPase from leaves is also subject to post-translational redox-regulation.

Using a new extraction procedure, day samples of leaves of pea, potato and Arabidopsis were found to contain more monomeric AGPB than samples harvested during the night. This conversion of the AGPB dimer into monomers is accompanied by an increase in AGPase activity, involving changes in the kinetic properties.

Light and sugar act as separate inputs to allow conversion of the inactive dimer into the active monomeric form. AGPB is rapidly converted into a dimer when leaves are darkened during the day. Moreover, monomerisation is induced in isolated chloroplasts by illumination. When leaves were fed sucrose during the night, AGPB is also converted from dimer to monomer, inducing a higher rate of starch synthesis. Starch deficient *pgm* mutants in which sugar levels are higher than in wildtype plants, showed a particularly marked conversion of AGPB from the dimeric to the monomeric form during the day. Plots of the extend of AGPB monomerisation against leaf sugars levels reveal a correlation between monomerisation during the diurnal cycle, with an increased monomerisation at a given sugar content in the light compared to the dark.

Thus, redox regulation allows starch synthesis to be regulated in response to light and sugar levels in the leaf, thereby complementing the regulatory network that coordinates fluxes of metabolites and recycling of phosphate during photosynthetic carbon fixation and sucrose synthesis. At present we are investigating whether other metabolic pathways are also subject to redox regulation in vivo.

Tiessen, A., Hendriks, J. H. M., Stitt, M., Branscheid, A., Gibon, Y., Farre, E. M. and Geigenberger, P. (2002). Plant Cell 14, 2191-2213

22 Completion of germination in Arabidopsis is delayed by knocking out peptide deformylase

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Initiation of protein translation in plant chloroplasts and mitochondria occurs with a formylated methionine. Peptide deformylase (DEF; Peptide deformylase; formyl-L-methionyl peptide + H_2O <= formate + methionyl peptide; EC 3.5.1.88) hydrolytically removes the N-formyl group cotranslationally. This essential process, exemplified by the lethality of *Escherichia coli* DEF knockouts, has recently been confirmed in eukaryotic organelles. Two peptide deformylases (*At*DEF1 and 2) with N-terminal chloroplast targeting sequences were identified by the Arabidopsis genome sequencing effort and the cloned forms of *At*DEF1 and 2 had peptide deformylase activity. To gain a better understanding of the functional significance of this activity, a reverse genetics approach was undertaken. Recovered mutant plants (α-population; University of Wisconsin Biotechnology Center's Arabidopsis Knockout Facility (http://www.biotech.wisc.edu/Arabidopsis/)) had a T-DNA insertion after the third codon of the gene for the full-length *At*DEF2 protein. Using non-cross reacting antibodies, the relative amounts of both *At*DEF1 and 2 were directly assessed. The absence of *At*DEF2 protein was apparently compensated by an upregulation of *At*DEF1 in the knockout plant relative to Wassilewskija (Ws; Russia) background, suggesting that *At*DEF1 may be functionally redundant to *At*DEF2 in the chloroplast. The seed of the *Atdef2*-knockout had a striking phenotype; 1000seed weight was significantly lower in the mutant as compared with Ws. The time to completion of germination with and without prechilling of the *Atdef2*-knockout seed relative to the Ws counterpart will be described in detail.

23 Slower growth and altered lipid metabolism in a mutant with reduced saturated fatty acids

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Acyl-acyl carrier protein thioesterases determine the amount and type of fatty acids that are exported from the plastids. To better understand the role of the FATB class of acyl-ACP thioesterases, we identified a T-DNA insertion mutant in the Arabidopsis FATB gene. Palmitate (16:0) content of glycerolipids of the mutant was reduced by approximately 50 % in all tissues and stearate (18:0) was reduced by 50% in leaves and by 30% in seeds. Growth rate was reduced in the mutant, resulting in 50% less fresh weight compared with wild-type plants. Furthermore, mutant plants produced seeds with low viability and altered morphology. Analysis of individual glycerolipids revealed that the fatty acid composition of prokaryotic plastid lipids was largely unaltered, whereas the impact on eukaryotic lipids varied but was particularly severe for phosphatidylcholine, with a 4-fold reduction of 16:0 and a 10-fold reduction of 18:0 levels. Total wax load of fatb-ko plants was reduced by 20% in leaves and by 50% in stems whereas total cutin load was mainly unchanged but its composition was reduced in derivatives of palmitate and increased in synthesis of C18:1 derivatives. Analysis of sphingoid bases indicated that the mutant cells maintained wild-type levels of these 16:0 derived components. Unexpectedly, labeling experiments revealed that despite a slower growth rate, leaves of fatb-ko plants synthesize fatty acids at a higher rate than wild-type leaves. Together, these results demonstrate the in vivo role of FATB as a major determinant of saturated fatty acid synthesis and that plants respond to reduced saturated fatty acids by slower growth but increased fatty acid synthesis.

24 Sizing the stem cell niche in the shoot meristem

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The stem cell population of the shoot meristem is reliably maintained although cells continuously leave the meristem and are replaced by new ones. We previously showed that the stem cells are maintained in a niche within the shoot meristem by signaling from an underlying organizing center, expressing the WUSCHEL gene (Mayer et al. 1998, Cell 95, 805-815), and that the size of the stem cell population is dynamically regulated by a feedback loop between stem cells and organizing center (Schoof et al. Cell 100, 635-644). This signaling circuitry appears to have the potential to act as a self-regulatory system, that is integrated into a larger regulatory network, controlling organ formation from the shoot apex. Here we address the mechanisms that regulate the size of the stem cell niche.

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25 New players in stomatal patterning

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The epidermis of Arabidopsis leaves and stems is simple, consisting of mainly three cell types: trichomes, stomatal guard cells and pavement cells. Pairs of guard cells flank a pore through which water and carbon dioxide are exchanged, and can change shape to module the width of this pore. The complex of guard cells, pore and subtending airspace is termed a stomate. The distribution of stomata in the epidermis is nonrandom, and guard cells normally obey several patterning rules: (1) they are formed through a stereotyped lineage of asymmetric divisions, (2) they are patterned locally so that two stomatal complexes are never adjacent to one another and (3) the overall numbers of stomatal complexes are controlled globally in response to environment cues. Local pattern is likely to involve classic cell-surface mediated signaling based on the identification of a receptor-like molecule, TMM [1] and a subtilisin protease, SDD1, that may process the signal [2]. In an extensive screen for mutants that violated any of the patterning rules, we identified 5 new loci that affect local pattern. One of these mutationally defined loci, *YODA*, encodes a component of a conserved MAP kinase signaling pathway and acts as a switch to control cell fate in the epidermis. In the absence of *YODA*, the majority of epidermal cells become stomata. Conversely, when an overactive or unregulated form of YODA is expressed in plants, no stomata are formed. The potential targets of this MAPK signaling pathway and *YODA's* relationship to the other known stomatal patterning genes will be presented.

1 Nadeau and Sack (2002) Science 296: 1697-1700; 2 Berger and Altmann (2000) G&D 17:1119-31

26 Chromatin Remodeling and the Maintenance of *knox* Gene Silencing

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Development in plants is dependent on a balance between the maintenance of stem cells in the shoot apical meristem (SAM) and the recruitment of daughter cells to form lateral organs. A key step that distinguishes organ founder cells from stem cells in the SAM is the down-regulation of the class I *knotted1*-like homeobox (*knox*) genes. In *Arabidopsis*, there are four class I *knox* genes; *STM*, *KNAT1*, *KNAT2* and *KNAT6*. As daughter cells are recruited from the SAM and lateral organs are initiated, *knox* gene expression must be down-regulated for proper leaf development. One of the genes responsible for maintaining *knox* gene silencing during leaf development is *ASYMMETRIC LEAVES1* (*AS1*). *AS1* is a myb domain transcription factor with homology to the maize *rough sheath2* (*rs2*) and *Antirrhinum PHANTASTICA* (*PHAN*) genes. Null mutations of *AS1*, *rs2* or *PHAN* result in the misexpression of *knox* genes in the developing leaf primordia.

In *rs2* null mutants the misexpression of *knox* genes results in an accumulation of overproliferating cells and displacement of sheath tissue distally into the blade. The misexpression of the *knox* genes occurs in a clonal pattern suggesting that the misregulation arises in the meristem. Since *rs2* is not expressed in the meristem its role is to maintain repression after the initial downregulation of the *knox* genes. To determine what other factors are involved in the repression of *knox* genes during leaf development, a yeast two hybrid screen was performed to identify proteins that interact with RS2.

Eight RS2 interacting proteins were verified. They include the maize homologues of ASYMMETRIC LEAVES2, RS2 itself and the chromatin remodeling factor HIRA. We are currently testing these interactions using the *Arabidopsis* homologues and have analyzed publicly available insertion lines to further characterize the interacting proteins. T-DNA insertions in the *HIRA* gene are lethal. Therefore, *Arabidopsis* 35S::*HIRA* lines were constructed. These cosuppression lines have a phenotype similar to *AS1* mutants. RT-PCR analysis confirmed that the 35S::*HIRA* lines are misexpressing *knox* genes. Based on these observations, we believe AS1 and AS2 form a complex that recruits HIRA to maintain a silenced chromatin state at the *knox* loci after daughter cells have been recruited from the SAM to form lateral organs.

27 Expression profiling of Arabidopsis cell types isolated by laser-capture microdissection

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Laser-capture microdissection (LCM) is a technique by which individual cells can be harvested from tissue sections while they are viewed under the microscope, by tacking selected cells to an adhesive film with a laser beam. Harvested cells can provide DNA, RNA, and protein for the profiling of genomic characteristics, gene expression, and protein spectra from individual cell types. We have optimized LCM for a variety of plant tissues and species, permitting the harvesting of cells from paraffin sections that maintain histological detail. RNA can be extracted from LCM-harvested plant cells in amount and quality that are sufficient for the comparison of RNAs among individual cell types. The linear amplification of RNA from LCM-captured cells permits the expression profiling of plant cell types, beginning with the isolation of from a few dozen to a few hundred cells. We demonstrate the capture and profiling of a variety of cell types from Arabidopsis.

28 Auxin Patterning and Organ Initiation in the Shoot Apical Meristem

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A fundamental developmental process in plants and animals is the positioning of lateral organs. In plants, recent experiments have implicated the hormone auxin in controlling this process. When polar transport of auxin is disrupted organs fail to initiate, and this can be rescued by exogenous auxin at the site of application (Okada et al., 1991; Reinhardt et al., 2000).

To gain a more detailed understanding of the role of auxin in primordium initiation we are using a combination of novel live-plant imaging techniques and genome-wide transcriptional profiling to examine auxin transport and auxin induced gene expression dynamics in the shoot apical meristem. Imaging of transporter-GFP fusion proteins and *in situ* expression analysis indicates that PIN1 and AUX1 are likely to transport auxin directly to sites of primordia initiation. Furthermore we show that these genes are themselves regulated by auxin, thus revealing a positive feedback loop that appears to concentrate auxin to localized domains. The auxin synthesis gene *NIT2* may also participate in this feedback loop since it also appears to be up-regulated by auxin.

Present experiments are aimed at relating the role of auxin with that of known patterning genes such as *FIL* and *LFY* and gauging expression responses to disruptions to auxin patterning. Preliminary data suggest that some of these genes may be indirectly regulated by auxin while others, such as *DRN*, are direct targets. We are constructing GFP reporters for these and other genes and verifying our micro-array data with RT PCR in an attempt to determine the spatio-temporal dynamics of their expression with respect to auxin action in the SAM.

Okada K., Ueda J., Komaki M. K., Bell C. J. and Shimura Y. (1991). Requirement of the auxin polar transport system in early stages of Arabidopsis floral bud formation. *Plant Cell* 3: 677-684.

Reinhardt D., Mandel T., Kuhlemeier C. (2000). Auxin regulates the radial position of plant lateral organs. *Plant Cell* 12: 507-518.

29 Using root hairs to dissect cell morphogenesis in plants

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The root epidermis is composed of hair cells which are located between two underlying cortical cell files and non-hair cells that are located over a single cortical cell file. Numerous labs have identified genes that regulate the patterning of cell types in the epidermis. These incude *TTG*, *WER*, *CPC* and *GL2*. We have recently shown that these genesare active during embryogenesis when epidermal pattern forms. These genes continue to act in the seedling where they are involved in the maintenance of pattern. The action of these genes together results in the expression of *RHD6* in root hairs where it promotes hair cell development. Our current model is that *RHD6* promotes the expression of genes that are active during hair cell formation. These include genes that are involved in diverse processes of cell growth. Some of these processes were anticipated. For example our analysis of *kojak* and *root hair defective1* mutations has highlighted the importance of the cell wall in defining cell shape. Other mutants have provided entirely novel insights into the process of cell growth. For example *RHD2* has highlighted the role of reactive oxygen species not only in tip-growing root hair cells but also in cells that undergo diffuse growth. Latest results will be presented.

30 The *PLETHORA* Genes Are Involved in Stem Cell Maintenance in the Root Meristem

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Stem cells continuously divide to renew themselves as well as to produce cells which differentiate into specific cell types. In the *Arabidopsis* root meristem, stem cells surround a group of mitotically less active cells called the quiescent center (QC). The QC contributes to stem cell maintenance by generating short-range signals which inhibit differentiation of the stem cells. By using the promoter trap approach, we identified a gene which is specifically expressed in the QC and the surrounding stem cells and designated this gene *PLETHORA1* (*PLT1*). *PLT1* encodes a member of the AP2/EREBP family of putative transcription factors. We obtained several independent insertion mutants for *PLT1* as well as for its closest homolog, *PLT2*. Each single mutant had only subtle defects in root meristem size and in cell division patterns of the QC and columella stem cells. However, the *plt1 plt2* double mutant displayed a striking phenotype in root meristem maintenance, as was revealed by highly reduced root meristem size and lack of columella stem cells. All meristem cells of the double mutant eventually differentiate as early as 5 days post germination. These results, taken together with the expression pattern of *PLT1*, indicate that *PLT1* and *PLT2* are redundantly required for stem cell maintenance of the root meristem either by promoting the activities of the QC and/or the surrounding stem cells. We are currently examining the relation of the *PLT* genes to other known pathways such as *SCARECROW* / *SHORT-ROOT* or auxin, and results from these experiments will also be presented.

31 CLE40, a protein functionally equivalent to the stem cell restricting signal CLV3, controls root development

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Continuous growth and development of plants is controlled by meristems that harbour stem cell pools. Division of stem cells and differentiation of their progeny is coordinated by intercellular signaling. In Arabidopsis, stem cells in shoot and floral meristems secrete CLAVATA3, a member of the CLE protein family that activates the CLV1/CLV2 receptor complex in underlying cells to restrict the size of the stem cell population. We found that CLE40 encodes a potentially secreted protein that is distantly related to CLV3. While CLV3 transcripts are confined to stem cells of the shoot system, CLE40 is expressed at low levels in all tissues, including roots. Misexpression and promoter swap experiments show that CLE40 can fully substitute for CLV3 to activate CLV signalling in the shoot, indicating that CLV3 and CLE40 are functionally equivalent proteins that differ mainly in their expression patterns. Analysis of cle40 mutants shows that wild-type expression levels of CLE40 are insufficient to contribute to CLV signalling. High level expression of CLV3 or CLE40 results in a premature loss of root meristem activity, indicating that activation of a CLV-like signaling pathway may restrict cell fate also in roots. The cellular organization of cle40 root meristems is normal, but mutant roots grow in a strongly waving pattern, suggesting a role for CLE40 in a signaling pathway that controls movement of the root tip.

32 Interaction of GA signaling with *SCARECROW* function in root meristem maintenance

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It has recently been shown that reintroduction of *SCARECROW* (*SCR*) expression only in the quiescent center (QC) of *scr* mutants is sufficient to rescue QC identity and indeterminate root growth.[1] Combined with previous reports, this suggests that in *scr* the lack of QC specification results in the gradual dissipation of the surrounding stem cell pool through differentiation. Thus the meristem is not maintained, and primary root growth eventually ceases.

Preliminary data suggest that gibberellin (GA) signaling modifies root meristem maintenance in *scr*. While GA treatment of wild-type seedlings has no apparent effect on root length, GA treatment of *scr* mutants significantly decreases final root length. Similarly, seedlings mutant for *SPINDLY* (*SPY*), a negatively acting component of the GA response pathway, show no apparent root-length phenotype.[2] Double mutants of *scr* and *spy*, however, have significantly shorter roots that do *scr* mutants alone. Interestingly, seedlings mutant for *SHORT-ROOT* (*SHR*), a gene known to act upstream of *SCR*, have a shorter final root length than do scr mutants, and that length appears to be unaffected by GA treatment.

We are currently analyzing the possibility that *SCARECROW-LIKE 3* (*SCL3*) may represent the link between *SCR* function and GA signaling. SCL3 is a member of the GRAS family of putative transcriptional regulators, as is SCR. In situ hybridization has shown that its expression overlaps with that of *SCR*.[3] Preliminary promoter-reporter analysis indicates that its expression decreases in distinct cell types in *scr* and *shr* mutant roots. We are currently working with the Salk T-DNA insertion line available for *SCL3* to test our ideas.

[1]Sabatini et al. (2003) Genes Dev. 17:354
[2]Swain et al. (2002) Plant Phys. 129: 605
[3]Pysh et al. (1999) Plant J. 18: 111

33 Epidermal cell fate specification in the Arabidopsis root epidermis involves early action of the two bHLH genes *GL3* and *EGL1*

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A central question in developmental biology is how cells become determined to adopt a specific cell fate. The Arabidopsis root epidermis provides an excellent system to study cell fate specification since two cell types, root hair cells and non-hair cells, arise in a distinct position-dependent manner. Several genes that influence this cell fate decision have been identified previously. For example, *TRANSPARENT TESTA GLABRA (TTG)*, encoding a WD40 repeat protein, and *WEREWOLF (WER)*, encoding a MYB protein, have been shown to act upstream of the homeodomain protein GLABRA2 (GL2) in promoting non-hair cell fate. CAPRICE (CPC), a MYB-like protein without a known activation domain, has been linked to the specification of the hair cell fate. Experiments employing transgenic Arabidopsis lines overexpressing the maize *R* gene, a *MYC*-like gene from the anthocyanin pathway, have led to the suggestion that a bHLH transcription factor might also participate in the root epidermal cell specification pathway. Here, we provide evidence for this assumption by demonstrating that two bHLH genes, *GL3 (GLABRA3)* and *EGL1 (ENHANCER OF GLABRA3)*, are involved in root epidermis cell patterning in Arabidopsis. Both genes are closely related to the maize *R* gene, and have recently been shown to be involved in trichome cell specification. Using mutant and overexpression line analysis, we show that GL3 and EGL1 affect epidermal cell specification at an early stage and act in a largely redundant manner. We also investigated the influence of GL3 and EGL1 on the expression of *GL2* and *CPC*. Our results suggest a model in which the bHLH proteins GL3 and EGL1 participate in both the suppression of the hair cell fate in the N-position and the promotion of the hair-cell fate in the H-position by regulating both *GL2* and *CPC* expression.

34 HEN1 and a microRNA in flower development

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We are interested in how an undifferentiated cell in the floral meristem adopts one of the four fates to become a sepal, petal, stamen, or carpel cell. AGAMOUS, a MADS domain transcription factor, acts to specify the identities of the reproductive organs (stamens and carpels), while AP2, an AP2 domain transcription factor, acts to specify the identities of the perianth organs (sepals and petals). AG and AP2 are antagonistic to each other and restrict each other to its domains of action within the floral meristem.

In a sensitized genetic screen in the *hua1-1 hua2-1* background, in which the *AG* pathway is partially compromised, we isolated three mutations in the *HEN1* locus that result in reproductive-to-perianth organ transformation such that *hua1-1 hua2-1 hen1* flowers resemble *ag* flowers[1]. *AG* RNA and protein levels are both reduced in *hua1-1 hua2-1 hen1* flowers as compared to those in *hua1-1 hua2-1* flowers, suggesting that *HEN1* is required for *AG* expression. We discovered that mutations in *HEN1* or *DCL1*, an Arabidopsis Dicer homolog, result in no or reduced accumulation of microRNAs, indicating that both genes are required for microRNA biogenesis[2]. These findings suggest that a microRNA(s) acts in flower development.

microRNAs appear to regulate their target gene mRNAs negatively through base-pairing interactions. One of the microRNAs we cloned, mir172 RNA, is highly complementary to a sequence in the mRNA of the floral homeotic gene *AP2*, suggesting that mir172 RNA regulates (probably negatively) *AP2* expression. Since *AP2* is known to repress AG expression, it is likely that *MIR172* acts positively in *AG* expression by repressing *AP2*. Therefore, we hypothesize that the reduced expression of *MIR172* in hen1 mutants causes elevated *AP2* expression and consequently reduced *AG* expression. Consistent with this hypothesis, a loss-of-function mutation in *AP2*, *ap2-2*, rescues the homeotic defects in *hua1-1 hua2-1 hen1-1* flowers. The regulatory role of *MIR172* in *AP2* expression and consequently in flower development will be discussed.

1. Xuemei Chen, Jun Liu, Yulan Cheng, and Dongxuan Jia. (2002). *HEN1* functions pleiotropically in *Arabidopsis* development and acts in C function in the flower. Development 129, 1085-1094.
2. Wonkeun Park, Junjie Li, Rentao Song, Joachim Messing, and Xuemei Chen. (2002). CARPEL FACTORY, a Dicer homologue, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*. Current Biology 12, 1484-1495.

35 Regulation of flowering time and floral meristem identity by microRNAs

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In an effort to identify novel Arabidopsis genes involved in flowering time, we characterized early- and late-flowering mutants from a DuPont activation tagging collection. One early-flowering mutant, *EAT-D*, was extremely early flowering and displayed additional floral meristem identity defects that were virtually identical to those seen in *apetala2* (*ap2*) mutants. Subsequent cloning of the activation tagged *EAT* gene identified a 1.4 kb noncoding RNA which recapitulated the phenotype when independently overexpressed. A microRNA, *mir172*, is located within the EAT RNA, and subsequent experiments confirmed that *mir172* is overexpressed in the *EAT-D* mutant. The apparent target of *mir172* is a family of *AP2*-like genes, including *AP2* itself. We have characterized the expression pattern of *mir172* and the effects of *mir172* overexpression on the *AP2*-like target genes. In addition, we present evidence that at least one of the *AP2*-like target genes, *RAP2.7*, also regulates flowering time. Overexpression of *RAP2.7* delays flowering, whereas a *rap2.7* T-DNA insertion mutant is early flowering. Taking account of all the data, we will present a model whereby the *mir172* microRNA promotes flowering by downregulating several *AP2*-like floral repressors.

36 Pollen tube growth and guidance is regulated by POP2, an Arabidopsis gene that controls GABA levels

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The *Arabidopsis POP2* gene encodes a transaminase that degrades gamma-amino butyric acid (GABA), a small molecule signal. The mutant flowers accumulate GABA, but not other GABA pathway compounds suggesting that the *pop2* phenotype is a direct consequence of GABA accumulation rather than downstream deficiencies. *pop2* mutants are self-sterile due to (i) reduced pollen tube elongation in the septum and (ii) misguided pollen tube growth during final stages of pollen tube growth. To understand how increased GABA levels results in these pollen tube behavior abnormalities, we performed *in vitro* pollen tube growth and *in vivo* GABA localization experiments. High GABA impairs tube growth *in vitro* , but lower levels stimulate migration. *pop2* tubes are hypersensitive to GABA-mediated inhibition, presumably because they cannot metabolize GABA. Consequently, although *pop2* tubes can successfully grow in a wild type septum, elevated GABA in *pop2* pistils causes reduced elongation of mutant tubes.Wild-type pistils have a steep GABA gradient between the septum and the micropyle, the pollen tube target. In mutant, despite elevated GABA levels, GABA gradient between the septum and the micropyle still exists. Wild-type tubes equipped with a functional *POP2* enzyme continually sense the gradient by degrading exogenous GABA and thus distinguish the micropyle from rest of ovule cells, in both wild type and *pop2* pistils. However, the mutant with a defective *POP2* enzyme cannot sense the gradient and consequently is not properly guided to the micropyle. The cumulative effect of these growth and guidance defects in *pop2* results in sterility. These results establish a novel role for GABA in pollen tube growth and guidance.

37 The REPLUMLESS (RPL) homeodomain protein specifies replum formation during *Arabidopsis* fruit development

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The *Arabidopsis* fruit takes the form of a seedpod with two seedpod walls or valves joined at the valve margins to a central replum and septum. When the fruit matures, the valve margins separate releasing the valves so that the seeds can be dispersed. Previous studies have shown that the FRUITFULL (FUL) MADS-box transcription factor specifies valve development while the SHATTERPROOF (SHP) MADS-box transcription factors specify valve margin cell identity. The *replumless* (*rpl*) mutant was identified in a screen for mutants that affect replum development. In *rpl*-mutant fruit, the replum fails to form and instead the *rpl*-mutant replum cells adopt characteristics of valve margin cells. However, only the outer layers of the *rpl*-mutant replum are affected. Molecular markers for the valve margin including *SHP* are ectopically expressed in the *rpl* replum. When this ectopic SHP activity is removed in the *rpl shp* mutant, replum formation is recovered indicating that RPL is not directly required for replum cell fate, but is instead required to limit *SHP* to the valve margin giving replum cells the opportunity to develop. The *RPL* gene encodes a homeodomain protein and a *RPL::RPL-GUS* construct shows expression in the replum. Therefore, we suggest that RPL acts in the replum to specify replum development in a large part by negatively regulating the *SH*P genes

38 A GATA-factor mediating axis formation in the early embryo

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Early development of Arabidopsis proceeds through an almost invariant pattern of division and expansion. Axis formation is apparent by the early globular stage, when cells in the lower tier of the pro-embryo expand along the apical/basal axis. Root formation is initiated at about the same time and involves the uppermost cell of the suspensor, termed hypophysis, which gives rise to the quiescent center. Both processes are dependent on the establishment of polar auxin transport. We have recovered mutations in a gene, designated MONOPOLE, that severely disrupt root and axis formation in the early embryo. The cells at the base of mutant embryos divide aberrantly producing fewer and irregularly arranged daughters. The cells of the suspensor, including the hypophysis, stop dividing after the 8-cell stage. In contrast to wild type, growth of the mutant embryos is dominated by the upper tier. A variable fractions of the mutants develops into complete and viable seedlings indicating that MONOPOLE is not generally required for establishing an axis and root. Genetic evidence suggests that MONOPOLE is a negative regulator of auxin responses. PIN4, a presumptive auxin efflux carrier implicated in the formation of an auxin sink, is expressed ectopically in the center of the pro-embryo. Furthermore, MONOPOLE mutations act as suppressors of MONOPTEROS mutations. MONOPTEROS is a positive regulator of auxin responses in the early embryo, and loss of MONOPTEROS function results seedlings without root or hypocotyls. Double-mutants embryos are in every respect similar to MONOPOLE embryos and, if they germinate, have a hypocotyl and root. The MONOPOLE gene product is similar to transcriptional regulators of the GATA family. Preliminary results suggest that MONOPOLE transcripts are transiently expressed in the cells of the pro-embryo during the early stages of embryogenesis. We propose that MONOPOLE temporarily inhibits auxin responses, most importantly the formation of a sink, in the pro-embryo. This would allow the hypophysis cell to create an auxin sink, which in turn would polarize the cells of the pro-embryo.

39 Enhancers of *tir1-1* define new factors required for SCF^{TIR1}-mediated auxin response.

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Previous genetic and molecular studies in Arabidopsis have defined the SCF^{TIR1} ubiquitin-ligase as a positive regulator of the auxin response pathway. In response to auxin, SCF^{TIR1} targets members of the Aux/IAA family of transcriptional regulators for ubiquitin-mediated proteolysis. To identify additional factors required for SCF^{TIR1} function, we mutagenized *tir1-1* seeds and screened for mutations that enhance the relatively weak *tir1-1* auxin resistance phenotype. We reasoned that the *tir1-1* mutation might sensitize the pathway, such that further perturbations would result in severe auxin response defects. We have recently cloned three of the genes defined by this mutant collection: *eta1* is a recessive allele of *AXR6*, which encodes the AtCUL1 subunit of the SCF^{TIR1} complex; *eta3* is allelic to SGT1b, a factor known to be required for SCF function in yeast and recently identified as a component of R gene mediated plant disease resistance; and *eta2*, a hypomorphic mutation in a novel regulator of SCF activity. Genetic and biochemical studies examining the roles of these factors in SCF^{TIR1}-mediated auxin response will be presented.

40 Brassinosteroid Signal Transduction in Arabidopsis

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The plant steroid hormoness brassinosteroids (BRs) play important roles in plant growth and development. BR is perceived by a cell-surface receptor that contains the receptor serine/threonine protein kinases BRI1 and BAK1. BR signal transduction involves a cytoplasmic kinase, BIN2, and nuclear factors, BZR1 and BZR2/BES1. BIN2 is a member of the GSK3/SHAGGY-like kinase family and negatively regulates BR responses. BZR1 and BZR2/BES1 are nuclear components that positively regulate BR responses. BZR1 mediates both BR-regulated growth response and feedback inhibition of BR biosynthesis. The BIN2 kinase directly interacts with and phosphorylates BZR1 in vitro and negatively regulates BZR1 accumulation in vivo. Experiments using a proteasome inhibitor suggest that the phosphorylated BZR1 is degraded by the proteasome and that a phosphatase is involved in the regulation of BZR1. BR treatment induces dephosphorylation and accumulation of the BZR1 protein. These results indicate that BIN2 phosphorylates BZR1 and targets BZR1 for degradation by the proteasome and that perception of BR by the cell surface receptor kinases leads to BZR1 dephosphorylation and accumulation most likely by inhibiting the BIN2 activity. We have identified several BZR1-interacting proteins, which include a putative DNA binding protein. Transgenic experiments indicate that this DNA binding protein is involved in BR responses. We have also identified a number of BR-regulated proteins using two-dimensional difference gel electrophoresis (2-D DIGE). A model of the BR signal transduction pathway will be discussed.

41 Signal integration during seedling photomorphogenesis

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Hormones are at the heart of all plant growth and development; yet, the molecular mechanisms integrating diverse cues into discrete morphological outputs are largely unknown. Auxin and brassinosteroids (BRs) are required for a wide variety of vital growth processes in plants, including the light response of emergent seedlings. In a wide range of tissues and species, auxins and BRs work synergistically to promote cell elongation. We are using a combination of physiological, genetic, and molecular tools to characterize BR-auxin synergism in Arabidopsis. In addition, we are using global gene expression analysis to further elucidate the overlapping response of auxin and BRs, as well as to identify factors regulated in response to both hormones. By exploiting the synergistic interaction of these two hormones, we hope to uncover new signal transduction components of both pathways, as well as elucidate their combined role in regulating differential growth responses during seedling development.

42 Regulation of EIN3/EIL1 transcription factors by ethylene

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The plant hormone ethylene regulates a variety of stress responses and developmental adaptations in plants. Differential gene expression has been implicated in many ethylene-mediated responses. The Arabidopsis ETHYLENE INSENSITIVE3 (EIN3) gene encodes a novel DNA-binding protein that acts to regulate gene expression in response to ethylene. However, little is known about the mechanism of activation of EIN3 in response to ethylene. Here we showed that EIN3 and its close homolog EIL1 play an essential role in mediating ethylene-regulated gene expression and morphological changes. ein3 eil1 double mutant showed complete ethylene sensitivity in various aspects of ethylene responses. Moreover, virtually all ethylene-regulated (induced or repressed) genes were found to be dependent on functional EIN3/EIL1 genes. EIN3 protein was able to directly bind to the promoters of several immediate target genes both in vitro and in vivo. A consensus sequence derived from several EIN3-binding motifs was found to be sufficient for the EIN3-dependent ethylene responsiveness. To gain insights into how EIN3/EIL1 proteins are regulated in response to ethylene, a construct of EIN3-GFP fusion protein was introduced into Arabidopsis plants. In wild-type plants, EIN3-GFP protein was accumulated and localized in nuclear speckles in the presence of ethylene. In ein2 mutant plants, however, EIN3-GFP protein was not detectable in nuclei even in the presence of ethylene. Thus, we monitored the levels of endogenous EIN3 protein under various conditions and in various ethylene response mutants. Our data indicate that EIN3/EIL1 are essential transcription factors mediating ethylene responses and ethylene acts to regulate EIN3/EIL1 function at a post-translational level.

43 The DVL Family of Small Polypeptides Regulate *Arabidopsis* Development

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Polypeptide signals are important in plant signaling pathways. There are six groups of plant polypeptides identified to date. Among hem, four groups have been shown to be involved in plant pathogen defensive response, cell proliferation, self-incompatibility and shoot apical meristem determination via different signaling pathways. The emerging significance of of the polypeptide involvement in regulating plant growth and developemnt gives rise to a challenging question - how to identify more polypeptide signals in plants. The limitation of conventional ways in identifying additional signaling peptides is mainly due to the enormous efforts required to either purify them from plant tissues or identify their genes through mutational analyses. Activation tagging is a new approach and has been proven to be an effective means in uncover gene function. A novel polypeptide - DVL1 (DEVIL1) - was identified in a screen for mutants with altered silique morphology in *Arabidopsis*. Overexpression of *DVL1* results in pleiotropic phenotypes in leaves, inflorescence and siliques. *DVL1* was not annotated in *Arabidopsis* genome. cDNA library screening revealed that *DVL1* has a 153-nucleotide open reading frame encoding a 51 amino-acid polypeptide which shares no significant similarity to previously identified proteins. Sequence alignment shows that *DVL1* belongs to a family of related genes that are limited to seed plants. Ectopic overexpression of each of five closely related *Arabidopsis DVL* genes causes similar phenotypic changes, suggesting functional redundancy in the *DVL* gene family. Together, these results show that the DVL family represents a novel class of small polypeptide controlling plant development.

44 Phototropism: An interactive network of multiple light and hormone-response pathways leading to changes in gene expression and cell elongation

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Because of their sessile nature, plants have evolved a number of strategies that involve reversible changes in morphology to respond adaptively to changes in their environment. The phototropic response, or directional curvature of organs in response to lateral differences in light intensity and/or quality, represents one of the most rapid and visually obvious responses of plants to changes in their light environment. Analyses of Arabidopsis mutants that affect various aspects of the phototropism have defined several molecular genetic components involved the development of phototropic curvatures. Based on these studies we have developed the following mechanistic model for phototropism in response by low fluence rate blue light (BL): First, the directional BL signal is perceived by phototropin 1, which in a physical complex with a novel plant-specific protein NPH3, then influences the activity and/or localization of an auxin efflux carrier(s). This proposed change in auxin transporter activity is believed to lead to the formation of a lateral gradient of auxin across the stem, with it accumulating on the "shaded" side away from the directional light stimulus. Next it is hypothesized that the increased level of auxin in the "shaded" side of the stem stimulates the proteosome-dependent degradation of MSG2/IAA19, a protein that otherwise heterodimerizes with and inhibits NPH4/ARF7, releasing a repressed state and allowing for NPH4/ARF7-dependent transcription of genes necessary for increased cell elongation on the "shaded" side which drives the differential growth response. The conditional nature of the *nph4/arf7*-null mutants suggests that additional ARFs (and likely Aux/IAA proteins) contribute to phototropism in a partially redundant fashion. The "output" from the phot1-induced pathway appears to be modulated by several additional "inputs", including three additional photoreceptors (phyA, cry1, and cry2) and two additional plant growth regulators (ethylene and brassinolide). In the case of phyA this modulation of phototropism likely occurs, at least in part, through nuclear events.

45 Genomic and physiological studies of early cryptochrome 1 action in blue light demonstrate roles for auxin and gibberellin

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Blue light inhibits elongation of etiolated *Arabidopsis thaliana* hypocotyls during the first 30 min of irradiation through a mechanism that depends on the phototropin 1 (phot1) photoreceptor. Thereafter, the cryptochrome 1 (cry1) photoreceptor is required for growth inhibition but phot1 is not. To identify genes responsible for the cry1 phase of growth inhibition, mRNA expression profiles of *cry1* and wild-type seedlings given 45 min of blue light were compared using DNA microarrays (Affymetrix 8K chip). Approximately 200 genes were expressed at a higher level and approximately 200 genes were expressed at a lower level in wild-type seedlings compared to *cry1* at the point of cry1-response incipience. The genes included kinases, transcription factors, cell cycle genes, cell wall metabolism genes, gibberellin (GA) synthesis genes, and auxin response factors. High-resolution growth studies support the hypothesis that genes in the last two categories were indeed relevant to cry1-mediated growth control. Inhibiting the last step of GA_4 biosynthesis (3-beta hydroxylation) with the chemical prohexadione (BX-112) restored wild-type response kinetics in *cry1* and completely suppressed its long-hypocotyl phenotype in blue light. Treatment with prohexadione plus GA_4 completely reversed the effects of the inhibitor, restoring *cry1*-like growth kinetics to the mutant. Treatment of wild-type seedlings with GA_4 was not sufficient to phenocopy *cry1* seedlings but co-treatment with IAA plus GA_4 produced *cry1*-like growth kinetics for a period of approximately 5 h. The genomic and physiological data together indicate that blue light acting through cry1 quickly affects the expression of many genes, a subset of which suppress stem growth by repressing GA and auxin levels and/or sensitivity.

This work is in press at *The Plant Journal*.

46 PIF1, a phytochrome-interacting bHLH factor, is involved in the regulation of the greening process in Arabidopsis.

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Plants can respond to their environmental light conditions through the phytochrome (phy) family of sensory photoreceptors. However the signal transduction mechanisms by which the phys control gene expression are unknown. Here we report functional characterization of PIF1 (*p*hytochrome *i*nteracting *f*actor 1). PIF1 is a member of the bHLH family of transcription factors with sequence similarities to the previously reported phytochrome interacting factors 3 and 4 (PIF3 and PIF4). However, in contrast to PIF3 and PIF4, that have stronger affinity for phyB and weaker affinity for phyA, PIF1 interacts specifically and strongly with the biologically active Pfr forms of both phyA and phyB with similar affinities. PIF1 can bind to a G-box DNA sequence motif present in various light regulated promoters. However, DNA-bound PIF1 cannot interact with phyA or phyB. PIF1 can activate transcription in a transient assay, and the transcriptional activation activity is downregulated in a phyA- and phyB-dependent manner. We have generated *pif1* T-DNA insertion lines, *PIF1* overexpression lines, RNAi and antisense lines to investigate the biological function of PIF1. Strikingly, *pif1* mutants show a bleaching phenotype if 4-5-day old dark-grown seedlings are transferred to white light. In contrast, PIF1 overexpression lines turn green if seedlings grown in far-red light are transferred to white light. These results are consistent with levels of protochlorophyllide in the mutant (higher) and overexpression lines (lower) respectively, relative to the levels in the wild type. Protochlorophyllide is an intermediate in the chlorophyll biosynthetic pathway that causes photooxidative damage in light when present in the free form. Taken together, these data suggest that PIF1 is involved in phytochrome-mediated control of the greening process in response to light.

47 Ionic Signaling, Integrating Root Responses to Touch and Gravity

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Plants are capable of perceiving a wide range of internal and environmental stimuli, with gravity being perhaps the most constant and pervasive of these signals. Gravity has profound effects on plant growth and development, with gravitropism being one obvious response to this stimulus. However, for the root, other stimuli such as light, gradients in nutrients and touch stimulation from physical obstacles in the soil also alter growth patterns to optimize exploration of the soil. We have therefore assessed the ionic signaling events in the root cap that might regulate such tropic growth in response to the gravity signal and how this gravisensory system interacts with responses to other stimuli such as touch. Laser ablation studies have mapped the most gravisensitive cells to the core of the columella region of the root cap. Ion sensitive versions of GFP have allowed us to monitor a rapid cytoplasmic pH transient upon stimulation by gravity. Blocking this transient using caged protons suggests the pH signal is required for gravity sensing/signal transduction. Concomitantly, the cell wall of the root cap becomes acidified around the sensory cells implying regulation of the H^+/ATPase at the plasma membrane is involved in generating these gravity-related pH signals. Consistent with a role for plasma membrane transport processes in gravity signaling, mutations in the AKT1 plasma membrane K^+ channel reduce gravity sensitivity. In addition, these gravity-related pH changes are suppressed in gravisignaling related mutants such as *pgm1* and *arg1*. However, touch stimulation of the root cap inhibits the pH-dependent gravity signaling events and reduces root gravity sensitivity and subsequent gravitropic response. This interaction of the touch and gravity response systems is a microtubule dependent process and appears to be mediated by a wave of elevated cytosolic Ca^{2+} that propagates from touch stimulated cells to the gravity sensing apparatus of the columella. These observations suggest a model whereby touch and gravity coordinate root growth through the interaction of pH and Ca^{2+} signaling events in the columella cells. The columella may therefore represent a site where many signals are integrated to control a common growth regulatory machinery. This work is supported by grants from NASA and NSF.

48 Genetic Analysis of High Temperature Tolerance in *Arabidopsis*

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Plants, like other organisms, have the ability to acquire thermotolerance rapidly, within hours, to otherwise lethal high temperatures. Even plants in their natural distribution range may experience high temperatures that would be lethal in the absence of this rapid acclimation. We are defining genes involved in acquired thermotolerance by several approaches: 1) isolating loss-of-function thermotolerance mutants, 2) screening existing mutants in stress-related pathways for defects in thermotolerance, 3) inhibiting candidate thermotolerance genes using dsRNA, 4) and identifying genes regulated during acclimation by microarray analysis. Seven loci (*HOT* loci) have been identified in a loss-of-function screen for acquired thermotolerance of hypocotyl elongation. The *HOT1* locus encodes the molecular chaperone Hsp101, providing direct evidence that this Hsp is required for thermotolerance. *hot1* mutants grow normally in the absence of stress, but show defects in thermotolerance of seed germination, hypocotyl elongation, survival of 10-day old plants, chlorophyll accumulation, and in vivo luciferase activity, indicating Hsp101 function impacts a wide range of plant processes. In contrast, the other six *hot* mutants only show a subset of these phenotypes. Notably, none of the other *HOT* genes maps to chromosomal positions containing Hsps or heat shock transcription factors, and all express wild type levels of Hsp101 and small Hsps. Both intragenic and extragenic suppressors of a *hot1* mutant allele have been isolated to define further Hsp101 function. Screens of existing mutants for defects in acquired thermotolerance reveals that 10-day old seedlings of *fad2*, *fad7/8*, *cad1*, a uvh sensitive mutant, *abi1*, *abi2*, and *abi3*, a respiratory burst oxidase mutant, and *nahG* transgenics all fail to acquire thermotolerance in response to a conditioning pretreatment. Of these, only *uvh6* shows a phenotype at the hypocotyl stage that could have been detected in our mutant screen. The growth-stage specificity of loss-of-thermotolerance in the mutants may indicate differences in the types of damage incurred during different growth stages. Overexpression and inhibition of small Hsp expression (by RNAi) in transgenic plants supports a role for the cytosolic small Hsps in development of tolerance to high temperatures. Initial microarray analysis of RNA from thermotolerant vs. non-thermotolerant plants identifies a unique subset of genes that may be key to recovery from stress.

49 Two Non-coding RNAs, *AtGUT15* and *AtCR20*, modulate the ABA response at high temperature in *Arabidopsis*

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Noncoding RNAs represent an emerging class of transcripts that lack significant open reading frames and encode RNA as their final product. Analyses of *AtGUT15* and *AtCR20* genes, members of a non-coding gene family indicate that the physiological functions of these ncRNAs are associated with the plant ABA signaling network. *AtGUT15* and *AtCR20* are members of a family conserved among monocots and dicots. These genes are devoid of phylogenetically conserved ORFs or any ORFs > 100 amino acids. In contrast, they possess a conserved secondary structure maintained throughout evolution by compensatory mutations. We have isolated homozygous T-DNA insertion mutants for both genes (*atgut15-1* and *atcr20-1*). In addition, a homozygous double mutant line (*gut15/cr20*) has been generated. All these mutant lines showed no obvious altered phenotype under normal growth conditions. However, the *gut15/cr20* double mutant exhibits hypersensitivity to ABA when grown at 30C. Neither single mutation alone has a marked effect on germination at any tested condition. Consistent with the temperature-dependent phenotype, the expression of *AtCR20* and *AtGUT15* is regulated by temperature at both the transcriptional and post-transcriptional level. The implications of these findings are: 1) The *AtGUT15* and *AtCR20* gene family appears to be a necessary component of the early stage of development under specialized conditions. 2) They share redundant functions, at least partially. 3) ncRNAs like *AtGUT15* and *AtCR20* can be used by plants as a sensor to interrelate temperature and ABA signaling networks for controlling growth and development in response to the shifting environment. The connection between ABA signaling and RNA metabolism is emerging through the identification of three RNA-binding proteins ABH1, HYL1, and SAD1. Mutations in the corresponding genes cause ABA hypersensitivity in Arabidopsis. A very intriguing scenario would be that some ncRNAs, like *GUT15* and *CR20*, interact with these RNA binding proteins and thereby modulate ABA signal transduction. It seems likely that the investigation of these ncRNA mutants could lead to the discoveries of novel posttranscriptional regulatory mechanisms.

50 ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis

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Imposition of sub-optimal temperatures results in induction of many genes some of whose expression is controlled by CBF family of transcription factors. Using a genetic screen of EMS mutagenised *CBF3* promoter-luciferase Arabidopsis plants a mutant was identified that had impaired cold stress induction of luciferase in these plants. Map based cloning revealed that this gene codes for a MYC- like bHLH transcription factor and was named as *ice1* (*inducer of CBF expression 1*). The *ice1* mutant plants had significant reduction in transcripts of *CBF* and many downstream genes, thereby resulting in reduced survival of these plants under freezing and chilling conditions. ICE1 is localized to nucleus and binds specifically to MYC recognition sequences of the *CBF3* promoter. Overexpression of ICE1 enhances expression of *CBF* and other downstream genes and improves freezing tolerance of the transgenic plants.

51 Root system diversity links drought sensing with developmental plasticity

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The developmental plasticity of plants enables them to adapt their morphology to a changing environment. Our lab is interested in identifying the molecular sensors of environmental change and the signaling pathways that guide developmental strategies in the adult plant. We hypothesize that these responses will differ in plants that have evolved in distinct environmental conditions. Exploiting this natural variation may be the best strategy for developing crops with optimized whole-plant responses to abiotic stress. With this in mind, we have undertaken an in-depth analysis of water stress effects on root system morphology in various Arabidopsis ecotypes.

We previously demonstrated that lateral root growth in *A. thaliana* var. Columbia (Col) is inhibited by simulated drought conditions. High osmotica specifically reduce the percentage of lateral root primordia that emerge to form lateral roots in Col seedlings. In contrast, we have found that lateral root emergence in *A. thaliana* var. Landsberg erecta (Ler) seedlings is insensitive to water stress. To identify the underlying genetic basis for this ecotypic difference, we scored lateral root growth in 100 Col x Ler recombinant inbred (RI) lines under three osmotic conditions. We identified three major effect quantitative trait loci (QTL) responsible for over 50% of the phenotypic variation in root system morphology (*EDG1-3*, for elicitors of drought growth). We used regression and multiple factor analyses to isolate environmental effects in the mapping data and found two classes of QTL. *EDG3* correlates with root system differences independent of osmotic conditions. In contrast, the effects of *EDG1* and *EDG2* were osmotic-dependent; thus the gene(s) responsible for these QTL may specifically act as osmotic sensors or response factors. F1 progeny of selected RI line backcrosses provide data consistent with the QTL predictions and suggest that at least two dominant repressors in Col regulate lateral root growth during water stress. We are currently developing near-isogenic lines to isolate the genes responsible for the *EDG* QTLs. The identity of these genes will provide insights into how plants evolve distinct mechanisms to coordinate environmental sensing with development.

52 Increased tolerance to oxidative stress by PMSR over-expression

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A consequence of oxidative stress (OS) is the damage to amino acid residues of proteins. Protein oxidation can lead to conformational changes which in turn can lead to inactivation and proteolysis. Even more than Cys, Met is the most susceptible amino acid to oxidation leading to the formation of the sulfoxide (MetOH). The enzyme peptide methionine sulfoxide reductase (PMSR) reduces MetOH back to Met. Met residues, and the associated PMSR, have been referred to as the "last chance" antioxidant. Five PMSR genes have been identified in Arabidopsis. One gene product (PMSR-1) is targeted to the chloroplast, while the others are localized in the cytoplasm. In all of the tissues examined, RT-PCR analyses show that all the genes are transcribed. To determine the role of PMSR in the OS response, we constructed Arabidopsis transgenic plants over-expressing either PMSR1 (OEX-1) or one of the cytoplasmic enzymes, PMSR3-(OEX-3). The susceptibility of the transgenic lines to different stress conditions were tested. Several independent lines with higher PMSR1 or PMSR3 expression than plants transformed with empty vector (CON) were obtained. We exposed 4 week-old plants to either 10 μM methyl viologen (MV), or 24 hours of 600 μmoles m^2 s^{-1} of light (HL), or 350 ppb ozone/4 days (O_3). The response of the transgenic seedlings to heavy metals (Ni, Cd, Cu, Zn and Mn) was also analyzed. The plastid over-expressing OEX-1 plants showed increased resistance to MV and HL when measured in by chlorophyll A fluorescence and photosynthesis. However OEX-1 plants were as susceptible as CON plants to O_3. In contrast, the cytosolic OEX-3 plants were more tolerant to O_3 than CON plants while still being more tolerant to MV. The root growth test showed that OEX-1 and OEX-3 plants were more tolerant to 100 μM Zn and to Mn concentrations up to 2.0 mM than CON plants. There were no differences between CON, OEX-1, or OEX-3 plants with the other metals. We propose that PMSR is indeed involved in the protection of plants against OS and that the enzymes have a role in response to a variety of stress conditions.

53 Chemical Defenses of Arabidopsis thaliana

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Plants synthesize a vast diversity of secondary metabolites. Current estimates start at 200,000 different compounds in the vascular plants. Arabidopsis is though to contain upwards of 5,000 different compounds. The function and identity of most of these is currently unknown. Current theory suggests that the secondary metabolites are critical for plant/environment interactions including defense against herbivores and pathogens. We are studying the role that chemical defenses play in protecting *Arabidopsis thaliana* against insects and necrotrophic fungi. Arabidopsis predominant known chemical defenses are Glucosinolates, Phenylpropanoids and the phytoalexin, camalexin. We are utilizing natural variation to identify genes critical for synthesizing the different compounds as well as test how structural variation impacts plant defense. All three compound classes are derived from amino acids or amino acid intermediates and their biosynthesis may be intricately linked. To test this hypothesis, we have developed a metabolite profiling procedure that can measure all three major defense classes and various phenolic acids in a single sample. We are using this procedure to understand how various herbivore/pathogen signals regulate the different compound classes and compounds within a class. This will allow us to investigate how Arabidopsis changes its chemical defense profile to protect against herbivore and pathogen attack.

54 Programmed Cell Death is Modulated by Ceramide Kinase in Arabidopsis

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Lipids from the sphingolipid pathway are known to regulate cell death and stress responses in animals. Among them, ceramides are believed to play an important role in mediating death signals. We find that ceramides are induced by pathogen attack and can elicit cell death in Arabidopsis protoplasts. In addition, we find that the Arabidopsis mutant *acd5* which harbors defects in ceramide modification shows a spontaneous cell death phenotype. Cell death in *acd5* shows apoptotic features, including chromatin condensation and TUNEL staining early in the cell death process. We cloned the ACD5 gene and found it encodes a protein with high similarity to a biochemically characterized ceramide kinase (CERK) from humans. Purified recombinant ACD5 protein has high specificity for ceramides but not other sphingolipids or diacylglycerol. As expected, the recombinant mutant enzyme had very little activity, and *acd5* mutant extracts have greatly reduced CERK activity. Furthermore, the *acd5* mutant accumulates ceramides and is more susceptible to killing by ceramide treatment. ACD5 gene expression is induced by virulent pathogen infection, suggesting that ceramide phosphorylation may modulate plant disease responses.

55 Inducers of Systemic Acquired Resistance Regulate NPR1 Function through Redox Changes

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NPR1 is an essential regulator of plant systemic acquired resistance (SAR), which confers immunity to a broad-spectrum of pathogens. SAR induction results in accumulation of the signal molecule salicylic acid (SA), which induces defense gene expression by activation of NPR1. However, the mechanism of NPR1 activation by SA is unclear. In this study, we found that in an uninduced state, NPR1 is present as an oligomer formed through intermolecular disulfide bonds. Upon SAR induction, a biphasic change in cellular reduction potential occurs, resulting in reduction of NPR1 to a monomeric form. Monomeric NPR1 accumulates in the nucleus and activates defense gene expression. Inhibition of NPR1 reduction using a inhibitor of the pentose phosphate pathway prevents defense gene expression, whereas mutation of Cys82 or Cys216 in NPR1 leads to constitutive monomerization, nuclear localization of the mutant proteins and defense gene expression. These data indicate that NPR1 is regulated by redox changes resulting from accumulation of SA after SAR induction. This result uncovered the mechanism underlying the activation of NPR1 in the SAR signaling pathway.

56 *pmr4*, a Mutant Lacking the Callose Defense Response, is Resistant to Powdery Mildew

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In order to better understand the mechanisms of both resistance and susceptibility of plants to biotrophic pathogens we have been studying a set of Arabidopsis mutants that have become resistant to powdery mildew (*Erysiphe cichoracearum*). We have identified one of these mutants, *powdery mildew resistant 4* (*pmr4*), as a callose synthase. Induced production of callose (a beta-1,3-glucan) at sites of pathogen attack is generally regarded as a plant defense response. *pmr4* plants produce dramatically reduced callose in response to both pathogens and wounding. Plants lacking an induced callose response might be expected to be highly susceptible to pathogens, but we find the opposite: *pmr4* mutants are resistant to at least three biotrophic pathogens, including *E. cichoracearum*, *E. orontii* and *Peronospora parasitica*. The lack of callose in *pmr4* plants appears to influence host defenses, and not fungal compatibility. Mutations in the salicylic acid (SA) defense signaling pathway are sufficient to restore compatible growth of the fungus in *pmr4* mutants. Consistent with this SA-dependency, microarray analysis reveals broad up-regulation of salicylate and pathogen responses in *pmr4* plants. Thus, paradoxically, while *PMR4* is responsible for the callose defense response to both pathogens and wounding, it also appears to negatively regulate SA defense pathways.

57 Expression of PR genes in response to broomrape, a root parasitic plant

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Broomrapes (*Orobanche* spp.) are parasitic plants that attack the roots of many important crop plants, causing large yield losses. Previous research using tobacco as a host has demonstrated that Egyptian broomrape (*O. aegyptiaca* Pers.) induced expression of genes in pathways leading to phytoalexin synthesis, while the pathogenesis-related (PR) gene *PR1a* was not induced. This led to the hypothesis that broomrape selectively avoids or represses defense pathways associated with PR protein production and systemic defense responses. *Arabidopsis thaliana* (Columbia) was used as a host to address this hypothesis, and expression of a range of PR genes was assessed in roots and shoots. *Arabidopsis* plants were grown in a hydroponic, polyethylene bag system, with treatments consisting of non-inoculated controls, plants parasitized by broomrape, and controls with roots subjected to mechanical wounding, jasmonic acid, or salicylic acid treatments. Analysis indicated that *PR1*, *PR5*, and *PDF1.2* genes were not expressed in *Arabidopsis* roots following any of the treatments, while *PR2* was only weakly expressed. In these roots *PR2* was clearly detected in non-inoculated, broomrape-parasitized, and jasmonic acid-treated tissues, but was not evident in wounded and salicylic acid-treated tissues. In contrast to roots, expression of all PR genes was detected in shoots of *Arabidopsis*. Interestingly, broomrape parasitism induced shoot expression of all four genes as compared to non-inoculated plants, which showed either no (*PR1*, *PR5*, and *PDF1.2*) or some (*PR2*) expression. This induction of PR genes far from the point of attack provides the first evidence that parasitism by broomrape has a systemic effect on host defense responses and contributes to our understanding of why these plants are susceptible to parasitism. In this case it does not appear that broomrape is specifically avoiding or repressing PR gene expression, but rather that roots are simply not set to use this defense. This work further demonstrates that defense gene regulation in *Arabidopsis* roots differs significantly from that of shoots.

58 A Future for Arabidopsis Research: Omics, Onics, Etics, Atics, and Plant Development

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To understand the mechanisms of plant development we need better descriptions of plant growth and development at all levels: subcellular, cellular, intercellular, and organismic. These descriptions must be causal analyses, not just a detailed listing of what occurs as a plant grows. The past decade of work on Arabidopsis, and the development of new methods in genetics, genomics, gene expression analysis and bioinformatics make it possible to establish in detail the exact patterns of gene expression that follow from activation of key developmental regulatory genes; causal analyses of gene network interactions are becoming possible. Advances in microscopy (photonics) and image analysis make it possible to determine the exact patterns of cell division and cell displacement that occur as shoots and organs develop, and to observe in parallel the expression domains of key regulatory genes. Once such information is in hand, the goal of making computational models of cells and cell-cell interactions that accurately represent the processes of plant development will be achievable. These models should, in the limit, behave just as do real plants, thus allowing us to say for the first time that we (or our computers) understand in detail how plants develop. The early progress of my laboratory toward these goals, and some of the preliminary models, will be described.

59 Evolutionary genetics of drought adaptation in *Arabidopsis*

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Drought stress is ubiquitous, often severe, and has likely played an important selective role in the evolution of plant growth, development, and physiology. Despite the importance of drought as a selective regime, we know surprisingly little about natural genetic variation underlying drought tolerance. We are using several approaches to study the genetics of drought adaptation in *Arabidopsis thaliana*. Our goal is to provide a detailed analysis of adaptive evolution at both the phenotypic and molecular levels using 1) screens of *A. thaliana* accessions to characterize genetic variation for drought-related traits, 2) genetic selection studies to test the importance of drought-related traits in plant performance 3) and quantitative trait locus (QTL) and linkage disequilibrium (LD) mapping of adaptations. These data will provide some of the first information on genes that function in natural drought adaptation, their genetic architecture (*i.e.* number of loci, magnitude of effects, patterns of interaction & pleiotropy), and will link molecular variation of known effect to evolutionary processes. Together, these methods will shed light on why, as well as how, the *Arabidopsis* genome has evolved with respect to functional variation in traits important in drought adaptation.

60 The evolution of expression patterns in the *Arabidopsis* genome

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Taking advantage of deep digital expression profiles from several tissues that include the majority of *Arabidopsis* transcripts, we are studying two different evolutionary issues concerning the evolution of expression patterns. First, variation in recombination rate along the chromosome is causally related to the efficiency with which natural selection can discriminate among neutral, deleterious and beneficial mutations. We ask what effect recombination rate has on patterns of expression and on regional patterns of coexpression. Secondly, little is known about the tempo of divergence in expression profiles among members of plant gene families. We ask what effect different modes of gene duplication have on patterns of divergence among paralogous genes. These studies shed new light on processes influencing the evolution of gene order and the diversification of gene families in *Arabidopsis*.

61 Molecular Evolution of the Receptor-Like Kinase Family in Arabidopsis and Rice

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Receptor-like kinases (RLKs) belong to a large gene family in Arabidopsis with more than 600 members that control growth, development and defense responses. We used a combination of bioinformatic and molecular evolutionary approaches to compare Arabidopsis and rice RLK family members. Surprisingly, the rice subsp. indica has nearly twice as many RLK family members as does Arabidopsis. This doubling is not a consistent feature in other gene families analyzed, indicating that the larger RLK family in rice is not simply a consequence of larger rice proteome size. Base on the phylogeny of all Arabidopsis and rice RLK members, we estimated that the common ancestor of Arabidopsis and rice had at least 372 RLK family members. After the split between Arabidopsis and rice, independent large-scale expansions occurred in both lineages in the past 160 Mya. Interestingly, RLKs involved in developmental functions tend to be found in subfamilies that duplicated little after the Arabidopsis-rice split. RLKs involved in disease resistance, on the other hand, are in general located in subfamilies with appreciable duplication events after their divergence. Similar proportions of RLK members are found in tandem repeats. But more large-scale duplication events were found in rice than in Arabidopsis in an analysis using the non-synonymous substitution rate as a proxy for time. These findings suggest a more prominent role of large-scale duplication events that may involve whole-genome duplications. The domain organizations of most RLK subfamilies were established prior to the Arabidopsis-rice split with notable exceptions. These exceptions demonstrate the modularity of RLK design that allows the attachment of novel extracellular domains (ECD) and the reuse of existing ones. Interestingly, ECDs tend to have higher Ka and more positively selected sites than those in intracellular domains (ICDs). The differences in the selection regime potentially reflect constraints on the ICDs for consistent signaling output and divergence in the ECDs to increase the spectrum of inputs.

62 The effect of outcrossing in a randomly mated population of *A. thaliana*

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A variable population of *A. thaliana* was produced artificially by randomly mating 19 world-wide accessions for four generations. Since *A. thaliana* reproduces mostly through selfing, it is possible that the original genotypes of the 19 accessions contain coadapted genes, causing the recombined genotypes to have a lower performance than the original accessions. On the other hand, the randomly mated population has higher heterozygosity, which is predicted to increase fitness. To evaluate the effect of recombination in life-history traits, the 19 original accessions and 96 genotypes from the randomly mated population were grown simultaneously in a growth chamber. During the plant growth and development of these plants many traits were measured such as: rosette growth, flowering time, number of fruits and seed production. As predicted the overall trend observed was that the randomly mated population has smaller growth and fitness. However, the differences were biologically small and only sometimes statistically significant. Comparison of F1 and parents suggest that the observed results are most likely due to recombination, although for some traits increased heterozygosity may explain the changes in phenotypic mean.

63 Attenuation of FLOWERING LOCUS C activity as a mechanism for the evolution of summer-annual flowering behavior in Arabidopsis

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Plant species have evolved a wide variety of flowering habits, each adapted to maximize reproductive success in their local environment. Even within a species, accessions from different environments can exhibit markedly different flowering behavior. In Arabidopsis, some accessions are rapid-cycling summer annuals, whereas others accessions are late flowering and vernalization responsive and thus behave as winter annuals. Two genes, *FLOWERING LOCUS C (FLC)* and *FRIGIDA (FRI)*, interact synergistically to confer the winter-annual habit. Previous work has shown that some summer-annual accessions contain null mutations in the *FRI* gene; thus it appears that these summer-annual accessions have arisen from winter-annual ancestors by losing *FRI* function. In this work we demonstrate that naturally occurring allelic variation in *FLC* has provided another route to the evolution of summer-annual flowering behavior in Arabidopsis. We have identified two summer-annual accessions, Da (1)-12 and Shakhdara, that contain functional alleles of *FRI*, but are early flowering due to weak alleles of *FLC*. We have also determined that the weak allele of *FLC* found in Landsberg erecta is naturally occurring. Unlike accessions that have arisen due to loss-of-function mutations in *FRI*, the *FLC* alleles from Da (1)-12, Shakhdara, and Landsberg erecta are not nulls; however, they exhibit lower steady-state mRNA levels than strong alleles of *FLC*. Sequence analysis indicates that these weak alleles of *FLC* have arisen independently at least twice during the course of evolution.

64 Auxin promotes Aux/IAA -SCF interaction through a soluble receptor

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Auxin response is mediated by the degradation of Aux/IAA proteins through the action of the ubiqutin-proteosome pathway. Recent studies have shown that auxin promotes the interaction between Aux/IAA proteins and SCF^{TIR1}. How auxin promotes this interaction is not understood. Similarly, the identity and location of the receptor responsible for auxin mediated response is unknown. To investigate the mechanism of auxin action, we tested whether auxin can promote the interaction between Aux/IAA and SCF^{TIR1} in a cell free system. Addition of auxin directly into protein extracts promoted the interaction between either GST-IAA7 or GST-IAA17 and SCF^{TIR1} suggesting that the auxin receptor and other signaling proteins are soluble factors. We tested several auxins including IAA, IPA, IBA, NAA and 2,4-D in our assay. While all active auxins promoted the interaction in a concentration dependant manner, IAA promoted interaction at a level as low as 0.05 nM. Phosphorylation and prolyl hydroxylation of substrates are two processes that promote interaction between substrates and E3 ligases. To test the possible role of phosphorylation and prolyl hydroxylation in the auxin-dependant interaction, we tested the kinase inhibitor staurosporine and the prolyl hydroxylase inhibitors Co2+, DMOG or DLP in our assay. None of these inhibitors interfere with the interaction suggesting phosphorylation or prolyl hydroxylation are not involved in this response. Previous studies demonstrated that two prolines in domain II of the Aux/IAA proteins are critical for interaction with SCF^{TIR1} and degradation. We therefore investigated the possible role of peptidyl-prolyl isomerase (PPIase) in this interaction. We tested the effects of three inhibitors, Cyclosporin A, rapamycin and juglone that affect, Cyclophillins, FK506 binding protein, and parvulins respectively. While Cylosporine A and rapamycin did not interfere with IAA7 - SCF^{TIR1} interaction, juglone strongly inhibited the auxin promoted interaction. Juglone also inhibited the auxin-induced degradation of NTAXR3-GUS in-vivo suggesting that a parvulin-type PPIase may play a role in this interaction. These results shed light on the important missing steps in auxin signaling.

65 SCD1 is required for stomatal cell cytokinesis and polarized cell expansion

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In the leaf epidermis, guard mother cells undergo a symmetric division to form the guard cells of stomata. We have identified a temperature-sensitive Arabidopsis mutant, *stomatal cytokinesis-defective 1-1 (scd1-1)* which affects this specialized division. Defective *scd1-1* guard cells are binucleate, forming incomplete cell walls. Under some conditions, such as in rapidly growing seedlings, cytokinesis is also disrupted in epidermal pavement cells. In addition, *scd1-1* affects cell division and expansion during root elongation. More severe *scd1* T-DNA insertion alleles, (*scd1-2* and *scd1-3*) exhibit greater reductions in polar cell expansion than *scd1-1*, observable in cells of the leaf epidermis and root, but most easily notable in trichomes and root hairs. All *scd1* alleles arrest early in floral morphogenesis, but *scd1-1* can make fertile flowers when grown at 16°C. *SCD1* is a unique gene in Arabidopsis. It encodes a protein related to animal proteins that interact with Rab GTPases (involved in intracellular protein transport) and/or mitogen-activated protein kinases. The subcellular localization of Scd1 was investigated using both a functional GFP-Scd1 fusion protein and anti-Scd1 antisera. By fluorescence microscopy, Scd1 is found at or near the plasma membrane in a punctate pattern. Fractionation studies from suspension-cultured T87 cells have demonstrated that Scd1 is a Triton-insoluble peripheral membrane protein. Further characterization of the distribution and function of Scd1 in vesicle trafficking during plant cytokinesis and cell expansion will be presented.

66 Histone acetylation/deacetylation implicated in embryonic polarity

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Arabidopsis embryos develop with a distinct apical/ basal polarity. Two groups of stem cells, the shoot apical meristem (SAM) and the root apical meristem (RAM), form at opposite poles of the embryo (the apical and basal poles respectively). *topless-1 (tpl-1)* is a single, temperature-sensitive mutation that transforms the shoot pole of the embryo into a second root pole. *tpl-1*, which was originally identified by Dr. Kathryn Barton, is the only Arabidopsis mutation identified to date that can cause this type of homeotic transformation of the embryo.

TPL encodes an 1131 amino acid protein containing 12 predicted WD40 repeats and a proline rich domain. These domains are also found in the TUP1/GROUCHO family of transcriptional corepressors. The N terminus of TPL contains a LISH domain that is also found in the corepressor LUENIG. *tpl-1* appears to be a gain of function allele that may act as a dominant negative for multiple TPL family members.

An unlinked suppressor of *tpl-1* was isolated and named *big top (bgt)*. *bgt* plants carry a mutation in the Arabidopsis homologue of the yeast GCN5 protein, a histone acetyltransferase. In yeast, GCN5 is recruited to chromatin by DNA binding transcription factors, where it acts as a transcriptional coactivator. *bgt;tpl-1* embryos develop normal apical structures and maintain the expression of apical genes, such as WUSCHEL, that are not expressed in *tpl-1* alone.

A mutation in an RPD3-like histone deacetylase, *ookpik (ook)*, enhances the embryonic phenotypes of *tpl-1*. Proteins of this class typically act as transcriptional corepressors in other systems. When grown at high temperatures (29 degrees), *ook* embryos display fused cotyledons similar to weak *tpl-1* phenotypes. *tpl-1* also becomes semidominant in an *ook* mutant background.

These results indicate that TPL likely encodes a transcriptional corepressor and works in a pathway utilizing histone acetylation/deacytlation to correctly pattern the apical/basal axis of the embryo.

67 CESG - An Arabidopsis Structural Biology Project

CESG
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The Center for Eukaryotic Structural Genomics (CESG) was founded at UW-Madison as a collaborative effort to develop critical technologies for determining three-dimensional structures of proteins rapidly and economically. CESG's primary goal is to extend knowledge of fold-function space, using the proteome of the model plant *Arabidopsis thaliana* as its initial focus. Several considerations led to the choice of the recently sequenced *Arabidopsis thaliana* genome as the initial focus for the Center?s activities. First, CESG?s structural biologists are of the opinion that new structures have the highest impact if they represent novel folds with known biological functions, novel functions, or known folds associated with a biological function novel to that fold. Although structures can sometimes provide clear indications of function, more frequently, additional genetic, biological, or biochemical information is required to elucidate function. Of the several sequenced genomes of higher organisms, *Arabidopsis* appears to be ideally suited to high-throughput functional genomics. CESG's software periodically analyzes and ranks the entire *Arabidopsis thaliana* proteome, consisting of ~29,000 open reading frames, to determine target priority. The top targets meeting selection criteria are carried forward through a defined strategy leading to structure determination by either X-ray crystallography or NMR spectroscopy. Bioinformatics is being used to select targets likely to open up important regions of conformational space or to elucidate novel fold-function relationships. CESG also considers candidate proteins of structural interest suggested by members of the *Arabidopsis* community. In addition, CESG is collecting information on proteins and protein regions that are unstructured, as a means for furthering our knowledge of the biological roles of protein dynamics. All completed structures and supporting data are deposited in timely fashion in publicly accessible databases. Progress at CESG is tracked at the Structural Genomics web site at PDB. Technology and products developed by CESG, including clones, expression vectors, and excess protein, are being made available to the scientific community. This project is supported by NIH grant P50 GM64598.

68 Confocal Microscopy and Laser Excision Microdissection/Chemical Analyses of Arabidopsis Lignifying Xylem and Interfascicular Fibers Genetically Altered in Lignin Monomer Composition: Evidence for Differential Control of Monolignol Polymerization

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Arabidopsis is a most attractive model plant system in which lignin assembly, given that its entire genome sequence is known and putative monolignol pathway multigene families identified. We have analyzed the patterns of lignin initiation and subsequent lignin assembly in different cell types (xylem and interfascicular fiber) in *Arabidopsis*, particularly with plants modified in their monolignol compositions. Using a combination of confocal microscopy and cell laser excision/ chemical analysis, it was established that the p-coumaryl alcohol rich lignin (so-called H-lignin) in plants down-regulated in cinnamate 3-hydroxylase was deposited only in the middle lamella and cell corners of the lignified interfascicular fiber cells, whereas it was more uniformly distributed throughout xylem cell walls; however, the latter lacked structural integrity. In plants producing sinapyl alcohol-derived lignins (S-lignins) via upregulation of ferulate 5-hydroxylase, the monomers were targeted mainly to secondary wall regions (between cell corners) in interfascicular fiber cells; the xylem cell wall lignin components, on the other hand, could not be distinguished from wild type. These data provide further evidence that even in transgenic plants with altered monolignol compositions, the H, G (coniferyl alcohol) and S lignins are still differentially targeted to precise regions of the developing wall in order to be polymerized at the appropriate lignin initiation site. This is further evidence for full biochemical control of lignin assembly, i.e. via monomer transport, oxidation and directed polymerization. Furthermore, the question of macromolecular lignin assembly using a wide range of non-monolignol precursors, including 2-methoxybenzaldehyde, feruloyl tyramine, etc., (Boudet, 1998) should monolignol supply be reduced has since been largely, if not fully, disproven (Anterola and Lewis, 2002).

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Anterola, A.M. Lewis, N.G., 2002. Phytochemistry 61, 221-294.
Boudet, A.M. 1998. Trends in Plant Sci. 3, 67-71.

69 Factors that control DNA methylation and gene silencing in Arabidopsis

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An endogenous Arabidopsis gene that is subject to cytosine methylation and silencing, *PAI2*, serves as a facile reporter in genetic screens for methylation mutations. The *PAI* genes encode an intermediate enzyme in the tryptophan biosynthetic pathway. In some Arabidopsis strains including WS, these genes are arranged as an inverted repeat (*PAI1-PAI4*) at one locus plus two singlet genes (*PAI2* and *PAI3*) at unlinked loci, and are densely methylated at both CG and non-CG cytosines across their regions of sequence identity. Only *PAI1* and *PAI2* encode functional enzyme, and only *PAI1* is expressed due to the presence of novel promoter sequences upstream of the methylated region. The *PAI2* gene is silenced by methylation of its proximal promoter sequences. To exploit *PAI2* as a reporter locus, we isolated a *pai1* missense mutant variant of WS. The *pai1* strain displays PAI-deficient phenotypes including blue fluorescence due to accumulation of a tryptophan pathway intermediate, yellow-green leaf pigmentation, reduced size, increased bushiness, and reduced fertility because the remaining functional *PAI2* gene is silenced by methylation. Forward genetic screens with this reporter yielded loss-of-function mutations in the CMT3 cytosine methyltransferase and the SUVH4 histone H3 lysine 9 methyltransferase, both classes of which primarily affect non-CG methylation on *PAI2*. Here we report the results of reverse genetic experiments testing mutations in other putative gene silencing factors, including other cytosine methyltransferases, other histone modifying enzymes, and factors implicated in RNA silencing and RNA-directed DNA methylation.

70 Histone H4 Acetylation Patterns Determined by Mass Spectrometry

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Acetylation of nucleosomal H4 is associated with gene activation. We have utilized a combination of electrophoresis and matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) to first identify histone H4, and then analyze its acetylation state in vivo. Nucleosomal histones from plants grown in liquid culture exhibit little acetylation. When these plants are treated with the histone deacetylase inhibitor sodium butyrate transient acetylation states are trapped, and bulk acetylation of histones increases, as demonstrated by changes in histone mobility on triton-acetate-urea (TAU) gels. MALDI MS analysis of H4 protein from butyrate-treated plants reveals additional mass peaks corresponding to the predicted masses of N-terminal peptides with one to four acetyl-lysines. Only 4 acetyl H4 peptides are observed out of all those predicted to occur upon random acetylation of the 4 most N-terminal lysines (K5, 8, 12&16). The identities of these 4 peptides support a model in which H4 is acetylated in a processive fashion, beginning with H4K16 and ending with H4K5. Mass spectra on individual bands from the TAU H4 ladder lend further support for this processive model.

This work was funded by NIH R01-GM60380 and by NSF Plant Genome grant DBI-9975930, “Functional Genomics of Chromatin” (http://www.chromdb.org/).

71 The Maize *Inhibitor-diffuse (C2-Idf)* mutation induces a post-transcriptional gene silencing response

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The maize *Inhibitor-diffuse (C2-Idf)* mutation is a dominant loss-of-function allele of *c2*. The *c2* gene product is chalcone synthase, the enzyme catalyzing the first dedicated step in the anthocyanin and phlobaphene pigment pathways. Without a functional *c2* product, no pigments are made. Plants that are heterozygous for wild-type *C2* and a recessive non-functional *c2* allele make half the normal amount of pigment. By contrast, plants that are heterozygous for wild-type *C2* and *C2-Idf* make virtually no pigment. We are interested in understanding how *C2-Idf* inhibits the function of a normal *C2* allele.

Experimental observations suggest that *C2-Idf* inhibition involves regulation at the epigenetic level, perhaps through an RNA-based silencing mechanism. *C2-Idf* homozygotes accumulate very little *c2* mRNA. In *C2-Idf/C2*heterozygotes, *c2* mRNA is reduced to roughly 10% of wild-type. This implies both a negative interaction between the inhibitor and wild-type alleles and that the loss of function associated with *C2-Idf* inhibition is controlled at the level of RNA accumulation. There are no major sequence changes between *C2* and *C2-Idf* in the coding and nearby flanking regions. However the promoter of the *C2-Idf* allele is hypermethylated relative to the wild-type, further suggesting an epigenetic effect on regulation of this allele.

As a means to test theses ideas, we sought to determine whether *C2-Idf* is regulated by a transcriptional or post-transcriptional mechanism. Nuclear run-on transcription revelaed that in heterozygous plants, *C2*-homologous transcripts are made. We infected *C2-Idf/C2* plants with maize dwarf mosaic virus, a potyvirus that carries an inhibitor of post-transcriptional gene silencing. In contrast to the unpigmented phenotype of uninfected plants, infected plants were highly pigmented. The increased pigmentation was accompanied by increased *c2* mRNA accumulation. These results suggest a post-transcriptional gene silencing mechanism as the mechanism as the means for *C2-Idf* of wild-type *C2*. This is one of few examples in plants of a naturally occurring mutation that is regulated through this mechanism.

72 Using Arabidopsis to study cytosine methylation

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Our group has been studying the regulation and function of cytosine methylation by combining different genetics approaches in Arabidopsis. One arm of this analysis has been characterization of mutations isolated by forward genetic screen for altered DNA methylation. We continued to focus our efforts on understanding the function of the DDM1 locus, which was identified in such a screen and which encodes a SWI2/SNF2-class nucleosome remodeling factor. More recently, we have begun exploiting natural variation in the different Arabidopsis strains to examine the prevalence, stability, and inheritance of epigenetic variation in cytosine methylation. Finally, as part of a larger NSF-funded genomics consortium (headed by Richard Jorgensen at the University of Arizona; 9975930), we have been investigating a collection of RNAi mutants in genes suspected to be involved in chromatin regulation (www.chromdb.org). This poster will present some examples of our current findings on these projects.

73 The HECT ubiquitin-protein ligase (UPL) family in Arabidopsis: UPL3 has a specific role in trichome development.

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Attachment of one or more ubiquitins to various intracellular proteins has a number of roles in plants including the selective removal of regulatory proteins by the 26S proteasome. The final step in this modification is performed by ubiquitin-protein ligases (or E3s) that promote ubiquitin transfer to appropriate targets. One important family of E3s is defined by the presence of a HECT domain, an active site first found at the C-terminus of the human E3, E6-AP. Using a consensus HECT domain as the query, we identified a family of seven HECT-containing ubiquitin-protein ligases (*UPL1-UPL7*) in Arabidopsis thaliana that can be grouped into four subfamilies. The *UPL3* and *4* subfamily encodes ~200-kDa proteins with four Armadillo repeats similar to those in the nuclear pore protein importin-α, suggesting that these E3s identify their targets through binding to nuclear localization sequences. Although T-DNA disruptions of the *UPL3* locus do not affect overall growth and development of Arabidopsis, the mutants show aberrant trichome development. Instead of developing three branches, many *upl3* trichomes contain 5 or more branches. The *upl3* trichomes also often undergo an additional round of endoreplication resulting in enlarged nuclei with ploidy levels of up to 64C. *upl3* plants are hypersensitive to gibberellic acid-3, consistent with the role of gibberellins in trichome development. The phenotype of *upl3* mutants is similar to that of *kaktus*, a previoiusly-described trichrome mutant with supernumerary branches. Allelism was confirmed by showing that *kaktus-2* plants harbor a splice site mutation within *UPL3*. Collectively, the data indicate that the ubiquitination of one or more activator proteins by UPL3 is necessary to repress excess branching and endoreplication of plant trichomes.

74 AtBRM, an ATPase of the SNF2 family, controls shoot development and flowering in *Arabidopsis thaliana*

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Activation and repression of gene transcription is mediated by changes in the structure of the chromatin. Ones established, cell type-specific pattern of gene expression must be stable over many cell generations and this is accomplished by labeling the chromatin (mostly DNA and histones) with epigenetic marks. ATP-dependent chromatin remodeling machines are involved in these processes and its role in plant development is an exciting new field. The first machinery of this type identified was the SWI/SNF complex from *Sacharomyces cerevisiae* and *Drosophila*. One of the protein subunits of the SWI/SNF complex is a DNA-dependent ATPase that is considered the enzymatic motor of the complex. These proteins were named SNF2 in yeast and BRAHMA in *Drosophila*. The brahma mutants in *Drosophila* show homeotic transformations and altered control of cell proliferation. We are investigating the role of AtBRM, an *Arabidopsis thaliana* homolog of Brahma. AtBRM contains the characteristics domains of the SNF2 subfamily of proteins: a DNA-dependent ATPase domain, a bromodomain and an AT-hook able to bind DNA inspecifically. AtBRM is a nuclear protein mostly expressed in inflorescences and in leaves. We have generated, by RNAi, *Arabidopsis* lines with reduced levels of the *AtBRM* mRNA. The *atbrm* plants present a pleiotropic phenotype characterized by small and downwards curly leaves, reduced height, closed flowers with small petals and early flowering in long-day.

75 Chromatin structure of T-DNA integration sites in Arabidopsis

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Controversy exists within the plant transformation literature regarding T-DNA integration into plant genomes. Uniform, genome-wide T-DNA insertion coverage depends upon the tacit assumption that insertion is random. Conversely, several papers have appeared that claim T-DNAs insert preferentially into transcriptionally active chromatin. Previous investigations of transgene integration may have been biased, because transgenic plants are typically identified by selection (usually based on antibiotic resistance). Under selective conditions, only plants that are capable of expressing a selectable marker are identified as transgenic. If a transgene is integrated into a region of transcriptionally inactive chromatin, the transgene may not be expressed, and thus the transformed plant may not be recognized. In order to circumvent this bias, we have used a pooled PCR strategy that allows us to identify transformed plants without requiring selectable marker activity. The activities of the reporter genes GUS and GFP in the "PCR-identified" plants and their progeny have been compared to those in "kanamycin-selected" lines. Preliminary data indicate that PCR-identified lines have a higher occurrence of silenced transgenes, suggesting that some integrated transgenes are not expressed. Transgenic plants that show little or no transgene activity have been identified, and the chromatin structures at the sites of integration are being analyzed.

76 Dimentional regulation of gene expression: from 1D to 2D & from 2D to 1D

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Promoters are regulatory sequences that control gene expression at the transcriptional level. A typical eukaryotic promoter consists of a minimal promoter and other upstream cis elements. The minimal promoter is essentially a TATA box region where RNA polymerase II, TATA-binding protein (TBP), and TBP-associated factors (TAFs) bind to initiate transcription, but it alone has no transcriptional activity. The cis elements, to which tissue-specific or development-specific transcription factors bind, individually or in combination, determine the spatio-temporal expression pattern of a promoter at the transcriptional level. The arrangement of upstream cis elements followed by a minimal promoter renders the promoter unidirectional, i.e., one promoter can direct the expression of one gene down stream of the TATA box region only. We have developed a strategy by which a unidirectional promoter can be bidirectionalized so that one promoter can direct two genes, one on each end of the promoter. On the other hand, the cis elements (sometimes called enhancers) of a promoter may affect neighboring gene activity. One example is the position effect of a transgene in plants. Eukaryotes have evolved a mechanism called genetic insulator to avoid potential "harassment" from neighboring genes. A dozen of genetic insulators have been identified from animals. We have recently identified a genetic insulator from Arabidopsis (the first of plant origin), and we have shown that this insulator can block gene expression when placed between a minimal promoter and cis elements/enhancers, and it has little effect on the promoter activity when placed outside of the promoter. The potential application of the dimensional control of gene expression in plants will be discussed.

77 Analysis of ASKs, Components of SCF Ubiquitin-Ligases

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The majority of targeted protein degradation in the cell is performed by the ubiquitin (Ub)/26S proteasome pathway. In this pathway, proteins to be degraded are covalently tagged with multiple ubiquitins by the sequential action of three enzymes, the E1, E2, and E3. The final enzyme in this process, the E3 Ub-ligase, binds the target and catalyzes attachment of the Ub moiety to the protein, which serves as a signal for degradation by the 26S proteasome.

One subfamily of E3 Ub-ligases is the SCF (**S**kp1, **C**ullin/**C**dc53, **F**-box protein) complex. More than 700 genes in *Arabidopsis* code for SCF components, including 21 *ASK* (*Arabidopsis Skp1-like*) genes. I am taking a reverse genetic approach to understand the function of the *ASK* genes in the plant. I have assembled 26 *Arabidopsis* T-DNA lines containing insertions in or near 13 *ASK* genes. In one example, I have identified a line (*ask16-1*) containing an insertion upstream of the *ASK16* coding region that results in over-expression of the gene. *ask16-1* appears normal under optimal growth conditions, but shows increased hypocotyl elongation in response to exogenous GA_3 treatment. Additional phenotypic characterization of *ask16-1* and a second allele, *ask16-2*, will be presented, as well as analysis of other *ASK* insertion lines.

78 Transcriptional network controlled by AGAMOUS during early stamen and carpel development

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Floral homeotic genes are assumed to initiate and maintain the programs of gene expression that lead to the development of each type of floral organ. In spite of the extensive genetic studies of floral development, very little is known about the downstream targets of floral homeotic genes. We have focussed on the C-function gene *AGAMOUS* (*AG*) that controls stamen and carpel development. We have monitored changes in gene expression during initiation and the early stages of stamen and carpel development. To achieve this, we generated an inducible AG system by making a fusion protein with the glucocorticoid receptor (GR) domain. Dexamethasone-dependent-rescue of reproductive organs was shown in *ag-3; AG-GR* plants, showing that AG-GR could replace AG function. When AG-GR is activated in the *cal-1, ap1-1* mutant background, reproductive organ primordia are initiated semi-synchronously, in quantities sufficient for transcriptome analysis. We then used whole-genome Affymetrix chips to reveal how gene expression changed one, three and seven days after AG-GR activation in *cal-1, ap1-1* meristems. Genes previously shown to function in carpel and stamen development (e.g. *AG* itself, *AP3*, *PI*, *CRC*, *SEP1*, *SEP2*, *SEP3*, *SHP1*, *SHP2* and *SUP*) served as positive controls and showed statistically significant increases during the time course. In addition to these positive controls, genes with previously unknown roles in organ development were identified. Activation of these candidate target genes by AG-GR was independently verified using RT-PCR. We have also confirmed AG-dependent expression in early stamen and carpel primordia in wild-type flowers by in situ hybridisation. Among the seven genes that were significantly activated throughout the time course, three suggest a role for gibberellin in early organ development. The function of these and other novel candidate AG targets will be discussed.

Session topic: Reproductive development

79 Common and distinct functions of the transcriptional co-activators GCN5 and ADA2 in Arabidopsis thaliana

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The histone acetyltransferase Gcn5 and an associated protein Ada2 are components of large transcriptional coactivator complexes in a wide range of eukaryotes. While the role of these factors in transcriptional control has been well studied in some experimental systems, relatively little is known about the function of these coactivators in plants. The Arabidopsis genome encodes one homolog of yeast Gcn5 and two homologs of Ada2, designated Ada2a and Ada2b. T-DNA disruption of *GCN5* or *ADA2b* results in severe effects on plant growth and development and on gene expression as monitored using DNA microarrays. The *gcn5* and *ada2b* mutant plants display many similar phenotypes, but other phenotypes are clearly different in the two mutants, suggesting that these proteins have both common and distinct biological functions. In contrast, disruptions of the *ADA2a* gene result in no dramatic phenotype under normal growth conditions. Overexpression of *ADA2a* fails to complement the *ada2b* mutation, suggesting that the Ada2a and Ada2b proteins are not functionally redundant. We are probing the distinct biological roles of the two *ADA2* homologs through double mutant analysis and localization studies utilizing promoter-GUS reporter fusion transgenes.

The biochemical activity of these transcriptional coactivators has also been assayed in several ways. GST pulldown and yeast two hybrid experiments show that Gcn5 can interact with both Ada2a and Ada2b. The ability of Gcn5 to acetylate free core histones in vitro is stimulated by Ada2a or Ada2b; furthermore, Ada2b facilitates the acetylation of nucleosomal histones by Gcn5. Interestingly, Gcn5 can acetylate both Ada2a and Ada2b in addition to histone H3, suggesting an autoregulatory mechanism. These in vitro acetylation sites have been mapped and their biological significance is currently under investigation.

80 AtGRF: A novel class of putative transcription factors interact with homologs of the human SYT protein in Arabidopsis

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The Arabidopsis Growth Regulating Factors (AtGRFs) contain two regions, the QLQ and WRC domains, in their N-terminal region and a transactivation domain in their C-terminal region. They constitute a novel class of putative transcription factors in seed plants, including OsGRF1 in rice. We demonstrated that AtGRF proteins are required for leaf and cotyledon growth. The QLQ domain shows high similarity to the N-terminal region of the yeast SWI2/SNF2 protein, which interacts with SNF11, another component of the SWI/SNF chromatin-remodeling complex in yeast. Therefore, the QLQ domain of AtGRF and related proteins is probably involved in protein-protein interaction as well. In search of partner proteins for AtGRF1 in Arabidopsis, we identified a novel gene family comprising three members and encoding putative transcription co-activators. Their human homolog, the SYT protein, interacts with a human SWI2/SNF2-type protein of the chromosome-remodeling complex. The Arabidopsis SYT1 protein (AtSYT1) formed nuclear speckles in onion epidermal cells as did human SYT. A series of deletion experiments showed that the QLQ domain and the C-terminal region of AtGRFs are involved in the interaction with the Arabidopsis SYT protein. The expression patterns of *AtSYT* genes are very similar to those of the *AtGRF* genes. Our results indicate that the AtGRF and AtSYT proteins form a functional complex that may be involved in leaf and cotyledon growth in Arabidopsis.

81 Chromatin modifications controlling nucleolar dominance

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Washington University

In an inter-species hybrid, the silencing of one parent's ribosomal RNA genes is an epigenetic phenomenon known as nucleolar dominance. In Arabidopsis suecica, the allotetraploid hybrid of A. thaliana and A. arenosa, and in diploid A. thaliana x A. lyrata hybrids, the rRNA genes derived from A. thaliana are selectively silenced by mechanisms that involve DNA methylation and histone deacetylation. Using RNA interference (RNAi) technology, we are systematically knocking down the expression of chromatin modifying enzymes and determining the consequences for nucleolar dominance. By targeting 13 genes representing the four families of histone deacetylases (HDAs), we have identified one HDA within the family related to yeast RPD3 and two plant-specific HDAs that are involved in rRNA gene silencing in nucleolar dominance. We have devised methods for obtaining pure nucleosomal histones and have identified all the core histones, including variants, using mass spectrometry. We have also identified the amino-terminal lysines of histones H3 and H4 that are acetylated and are determining the substrate specificity of the HDAs that enforce nucleolar dominance. Interestingly, knocking down a variety of chromatin modifying activities, including HDAs, has little phenotypic consequence in A. thaliana but causes dramatic developmental abnormalities in hybrids, suggesting that epigenetic mechanisms may be key to hybrid sucess.

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82 *PIE1*, an ISWI Family Gene, Is Required for *FLC* Activation

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Proper control of the floral transition is critical for reproductive success in flowering plants. In Arabidopsis, *FLC* is a floral repressor upon which multiple floral regulatory pathways converge. *PHOTOPERIOD INDEPENDENT EARLY FLOWERING1 (PIE1)* is required for the activation of *FLC* by *FRI* and also for *FLC* activation due to mutations in the autonomous pathway. PIE1 is similar to ATP-dependent, chromatin-remodeling proteins of the ISWI and SNF2/SWI2 family. The role of *PIE1* as an activator of *FLC* is consistent with the general role of ISWI and SNF2/SWI2 family genes as activators of gene expression. *PIE1* also has a role in petal development as revealed by the suppression of petal defects of the *curly leaf (clf)* mutant by the *pie1* mutation. Thus, the suppression of a PcG mutant phenotype (*clf*) by a trxG mutation (*pie1*) is observed in petal development.

83 Hypersensitivity of genetic hybrids to chromatin perturbations

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Using RNA interference (RNAi), we are knocking down the expression of chromatin modifying genes in *Arabidopsis thaliana*, in the allotetraploid species *A. suecica* and in diploid *A. thaliana* x *A. lyrata* hybrids. Interestingly, targeting chromatin genes by RNAi frequently causes no abnormal phenotype in *A. thaliana* but has dramatic phenotypic consequences in allotetraploids or in diploid hybrids. For instance, *A. thaliana* plants harboring the histone deacetylase HDT2 RNAi mutation appear phenotypically normal. By contrast, in *A. suecica*, whose genome contains diploid chromosome complements from *A. thaliana* and *A. arensoa*, the same RNAi construct causes pleiotropic abnormalities in leaf, inflorescence and floral morphologies and loss of the vernalization requirement for flowering. Similarly, *A. thaliana* HDT2-RNAi lines that show no abnormal phenotypes can be crossed to *A. lyrata* to produce F1 offspring that show phenotypic abnormalities similar to those observed in *A. suecica* HDT2-RNAi lines. Aphenotypic *A. thaliana* chromatin RNAi lines targeting numerous other chromatin genes also give rise to hybrid progeny with mutant phenotypes. These observations suggest that chromatin modifications are key to hybrid success, possibly by suppressing aspects of "genomic shock" first postulated by McClintock to occur in hybrids resulting from wide crosses.

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84 MicroRNA regulation of PHABULOSA and PHAVOLUTA expression

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PHABULOSA (PHB) and PHAVOLUTA (PHV) are two closely related class III HD-ZIP proteins with roles in the adaxial/abaxial patterning of leaves. They are two of the first predicted regulatory targets of microRNAs (miRNAs), endogenously expressed ~21 nucleotide RNAs thought to act as regulators of gene expression in both plants and animals. Gain-of-function *phb* and *phv* mutations not only alter the protein sequence of a potential sterol/lipid biding domain but also disrupt sequences complementary to miRNA165 and miRNA166. We are using two sets of experiments to determine whether the dominant phenotype caused by these mutations is the result of changes in protein function, microRNA regulation, or both. First, we have specifically disrupted the microRNA complementary site of the PHB cDNA with silent mutations. Transgenic plants expressing 35S:PHB display wild-type development while transgenic plants expressing 35S:PHB with these silent mutations have altered leaf development. Our data support a model in which microRNA regulation of PHB mRNA expression is essential for proper leaf patterning. For a second method, we have constructed compensatory mutations in miR165 genomic fragments to test for complementation of a *phv* gain-of-function mutant. The change in the miR165 sequence restores pairing with the altered PHV mRNA of *phv-1d*, a point mutation in the miRNA complementary site which results in a G to D change in the protein sequence. We are assaying the ability of transgenes containing these compensatory miR165 mutations to suppress the *phv-1d* phenotype.

85 Microarray analysis of germinating pickle seedlings

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Germination in angiosperms represents an important developmental transition where embryonic identity is repressed and vegetative identity emerges. The CHD3-chromatin remodeling factor PICKLE (PKL) is required for the repression of embryonic characters in germinating Arabidopsis seedlings. To identify genes that may be involved in promoting embryonic identity, a combined candidate gene approach and microarray analysis was used to identify genes that exhibit robust PKL-dependent repression of expression during germination. Several of the genes identified as having robust pickle-dependent transcript levels have expression patterns correlated with zygotic or somatic embryogenesis-including all three *leafy cotyledon* (*LEC*) genes. The suite of genes derepressed in *pkl* mutants during germination may promote the establishment of embryonic identity found in the *pkl*mutant. An analysis of recent data gathered using the Affymetrix whole-genome array will be presented and discussed.

86 The SUMO Modification System in *Arabidopsis*

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Small Ubiquitin-like MO difier (SUMO) is a member of the superfamily of ubiquitin-like polypeptides that become covalently attached to various intracellular target proteins as a way to alter their function, location, and/or half-life. Here we show that the SUMO conjugation system operates in plants through a characterization of the *Arabidopsis* SUMO pathway. An eight-gene family encoding the SUMO tag was discovered as were genes encoding the various enzymes required for SUMO processing, ligation and release. A diverse array of conjugates could be detected immunologically, some of which appear to be SUMO-isoform specific. The levels of SUMO1 and 2 conjugates but not SUMO3 conjugates increased substantially following exposure of seedlings to stress conditions, including heat shock, H_2O_2, ethanol, and the amino acid-analog canavanine. The heat-induced accumulation could be detected within 2 min from the start of a temperature upshift, suggesting that SUMO1/2 conjugation is one of the early plant responses to heat stress. This accumulation was desensitized to repeated heat shocks, indicating that either the substrates become limiting or the conjugation system is deactivated following the first shock. Over-expression of SUMO2 enhanced both the steady state levels of SUMO2 conjugates under normal growth conditions and the subsequent heat-shock-induced accumulation. This accumulation was dampened in an *Arabidopsis* line engineered for increased thermotolerance by overexpressing the cytosolic isoform of the HSP70 chaperonin. Knockouts in SUMO1 and 2 have been generated recently; their response to stress is currently under investigation. In addition, kockouts in genes encoding other components of the pathway have been identified and are currently being analyzed. Taken together, the plant SUMO1/2 conjugation pathway appears to have an important role in stress protection and repair possibly by affecting the activity and/or half-life of key stress regulators.

87 Transcript level-mediated gene silencing in plants

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Predictable, stable transgene expression is often a critical parameter for the broad use of transgenic plants. Unfortunately, transgene expression among independent lines transformed with the same construct often varies more than 100fold and gene silencing is frequently observed.

Single-copy T-DNA lines harbouring reporter genes of various kind and number under the control of a strong promoter were established in Arabidopsis for a comprehensive analysis of transgene expression. Characterisation of 132 independent transgenic lines revealed no case of silencing due to site of T-DNA integration. Below a certain number of identical transgenes in the genome, gene copy number and expression were positively correlated. Expression was high, stable over all generations analysed, and of a comparable level among independent lines harbouring the same copy number of a particular transgene. Conversely, silencing was triggered if the transcript level of a transgene surpassed a gene-specific threshold. Hallmarks of post-transcriptional gene silencing were observed in silenced lines: small interfering RNAs, methylation of the transcribed transgene region and meiotic reversibility. Transgene transcript levels above a gene-specific threshold, not position effects, trigger silencing in T-DNA transformants. Transcript level mediated silencing effectively accounts for the pronounced transgene expression variability seen among transformants.

88 Novel approaches to identify proteins interacting with the *Arabidopsis* Polycomb-group protein CURLY LEAF

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Polycomb-group (Pc-G) proteins play important roles in plant and animal development and maintain repression states of homeotic genes. In *Drosophila* Pc-G proteins were shown to act in large complexes.

The *Arabidopsis* Pc-G mutant *curly leaf* (*clf*) has a dwarf phenotype, is early flowering and its flowers show homeotic transformations. The *embryonic flower*2 (*emf*2) mutant shows an even stronger phenotype bypassing the vegetative state. A third Pc-G protein, FERTILIZATION INDEPENDENT ENDOSPERM (FIE) is also required for the repression of the transition to flowering.

To explain the partially overlapping phenotype of *clf*, *emf2* and *fie* two-hybrid analyses were carried out and showed that CLF may interact with FIE. Additionally, weak *emf*2 mutants have a similar phenotype to strong *clf* mutants and weak *clf* and *emf*2 alleles show a synergistic interaction in double mutants displaying no vegetative growth, thus a strong *emf*2 phenotype. Therefore, not only the proteins, but at least part of the complexes seem to be conserved between *Drosophila* and *Arabidopsis*.

The fact that *clf* and *emf*2 show different strength in their mutant phenotypes may be explained by the partial redundancy of CLF and the CLF homologous protein CLF-like (CLK). *clf clk* double mutants display an enhanced *clf*, *emf*2-like mutant phenotype although *clk* single mutants are aphenotypic.

To identify novel interaction partners of CLF different *in vivo* approaches are carried out. The nuclear yeast two- and three-hybrid and the cytoplasmic yeast Split-Ubiquitin systems are applied with CLF lacking the SET domain as the bait. As prey different cDNA libraries fused to the GAL4-activation domain or to the N-terminal part of UBIQUITIN are used. In the three-hybrid analyses FIE is co-expressed to identify proteins that only interact with the CLF/FIE complex. Both the use of the CLF truncation lacking the putative histone methyltransferase domain and the Split-Ubiquitin system are indicated as it cannot be excluded that full-length or nuclear CLF, in a complex with another protein, exhibits its histone methyltransferase activity in yeast, thus leading to lethal phenotypes.

89 Chromatin modulation and lifespan alteration in *Arabidopsis*

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Epigenetic mechanisms, such as changes in DNA methylation and histone acetylation, alter the expression of many different genes in an age-dependent and tissue-specific manner. The long-term goal of my research is to identify the genes and pathways responsible for chromatin modulation that alter lifespan. Among the most significant observations that link chromosome structure to lifespan alteration is the finding that elevated levels of SIR2, a yeast histone deacetylase, dramatically extends lifespan in yeast and worms. In yeast, SIR2 mediates silencing at various chromosomal loci; one effect of Sir2-mediated silencing is a repression of recombination of rDNA. This repression of genome instability delays the formation of ERCs (extra rDNA circles) which ultimately lead to the demise of aging mother cells. Interestingly, the finding that ERCs in yeast lead to the death of aging mother cells is reminiscent of a finding in the filamentous fungi Podospora anserina and Neuropspora crassa— senescence is always associated with the accumulation of defective mitochondrial DNA. Long-lived mutants show little if any disappearance of intact mitochondrial chromosome.

Arabidopsis provides a unique and unexploited system for the study of chromatin modulation in lifespan determination. First, *Arabidopsis* like other model organisms, has a small genome, a short, defined lifespan of 8-10 weeks, and a lifespan that can be extended by mutations, including those that cause defects in reproduction. However, the *Arabidopsis* system provides unique advantages. For example, development in *Arabidopsis*, like many plants, occurs in a progression along the length of the stem. New tissues are made throughout the life of the organism allowing direct comparisons of young and old tissue in the same individual. Also, *Arabidopsis* cells can be cultured and used to regenerate entire plants, allowing chromosome structure and gene expression studies in cultured cells, specific organs, or entire organisms. Finally, plants, unlike animals, tolerate huge aberrations in chromosome behavior and number, an enormous advantage as this allows the study of viable organisms undergoing extreme genomic instability. Our analysis includes studies of global methylation and acetylation/deacetylation patterns, isolation of mutants with altered lifespan, and analysis of Sir2.

90 FY is an 3′ end RNA processing factor that promotes flowering with FCA

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FCA is a novel, nuclear RNA binding protein that promotes flowering through the autonomous pathway. FCA expression is regulated in a complex manner: first, translation initiates at a non AUG codon and second, *FCA* pre-mRNA is alternatively spliced and polyadenylated at two sites. We have discovered that FCA protein interacts with another protein of the autonomous pathway, FY, a WD repeat protein that is highly related to an RNA 3′-end processing factor identified in yeast. Consistent with this sequence similarity, we have found defects in 3′ end formation in *fy* and *fca* loss-of-function mutants. We have also discovered that FCA autoregulates its expression by promoting premature 3′ end-formation within its own pre-mRNA. This explains the basis and regulatory significance of the alternatively processed *FCA* transcripts. FCA protein interacts with FY to autoregulate its expression, effectively pre-setting levels of available active FCA protein. FCA then interacts with FY in order to control *FLC*expression and hence, flowering time. We do not yet know if the regulation of *FLC* by FCA-FY is direct. To address this genetically, we have isolated suppressor mutants exhibiting elevated levels of *FLC* mRNA in a *35S::FCA* background. FCA-FY do not regulate *FLC* via other autonomous pathway components (eg., *FVE*, *FPA*) and conversely neither do these appear to regulate *FLC* via FCA-FY. The autonomous pathway may therefore represent the evolution of divergent mechanisms acting to control the expression of the critical repressor of Arabidopsis flowering,*FLC*.

91 Trichostatin A arrests seedling development in *Arabidopsis thaliana*

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Histone acetylation plays an important role in the regulation of gene expression. Histone acetylation is controlled by histone acetyl transferases and deacetylases, which are associated with activation and repression of gene expression, respectively. We have been studying the role of histone deacetylase during development in the model plant, *Arabidopsis thaliana*. We used Trichostatin A (TSA) to block histone deacetylase activity in germinating *Arabidopsis* seeds. TSA delayed the onset of seed germination by 1 day but the final percentage germination was the same as untreated control seeds. However, seedling development after germination was arrested in the presence of TSA in a dose dependent manner. Gene chip arrays were used to assess differences in gene expression in TSA-treated and untreated germinated seeds. A number of embryonic genes are up-regulated in TSA germinated seeds compared to controls indicating that histone deacetylase may be involved in repressing embryonic genes during the switch from embryonic to post-germinative development.

92 A reversible histone code is associated with gene regulation and development in *Arabidopsis*

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Disruption of *Arabidopsis thaliana* histone deacetylase 1 (*AtHD1*) expression induces developmental pleiotropy in *Arabidopsis*, indicating that AtHD1 either directly or indirectly affects gene expression. Genome-wide changes of gene expression in *AtHD1* disruption lines are analyzed using AFLP-cDNA display and oligo-gene microarray containing 26,090 70-mer oligos. Here show that over 500 genes are either up- or down-regulated in the *athd1-t1* line with a T-DNA insertion in *AtHD1*. Significantly, the expression of many homeotic genes important to plant development is affected; consistent with the developmental abnormalities observed in the *AtHD1* disruption lines. Furthermore, H3 Lys9 and H4 Lys12 are hyper-acetylated in the *athd1-t1* line, whereas H3 Lys9 is hyper-methylated and H4 Lys5 hypo-acetylated. Chromatin immunoprecipitation (ChIP) assays are employed to determine the association of a specific acetylation or methylation profile with activation or silencing of candidate genes. Results from this study provide direct evidence for the role of a reversible histone code in the control of plant gene regulation and development.

93 *Arabidopsis* Homologs of the Cleavage & Polyadenylation Specificity Factor

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The mechanism of mRNA 3[prime]-end processing in plants is not well understood. Using functional genomic tools, we have begun to study the genes and their encoding proteins that are relevant to nuclear mRNA polyadenylation. A set of *Arabidopsis* genes encoding homologs of the subunits of the mammalian cleavage & polyadenylation specificity factor (CPSF) complex was identified and termed *AtCPSF160, AtCPSF100, AtCPSF73-I, AtCPSF73-II* and *AtCPSF30*, respectively. These genes are widely expressed throughout the *Arabidopsis* plant, with the exception of *AtCPSF73-II* that is expressed mostly in flower tissues. Yeast two-hybrid analyses were used to examine all possible pair-wise interactions among these proteins, and the resulting protein-protein interaction profile is rather comparable to those characterized in mammals. These results suggest that the proteins encoded by these genes form CPSF complex in *Arabidopsis*, thus they may be involved in plant mRNA polyadenylation. In addition, we found that both overexpression and under-expression (inducible gene silencing) of *AtCPSF73-I* caused lethality in *Arabidopsis*, which suggest that the gene may be essential in plant growth and development, and under the control of a fine regulatory mechanism in plants. Moreover, interruption of the *AtCPSF73-II* gene resulted in a lethal phenotype in *Arabidopsis*, and the heterozygous mutant showed impairment of embryo development. *AtCPSF73-II*, homolog of which has not been found in yeast, seems to represent a new group of genes encoding a subunit of CPSF that is important for the development of multicellular organisms.

94 Regulation of gene expression by Arabidopsis HD2 histone deacetylases

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The four HD2 proteins of Arabidopsis thaliana (AtHD2A-D) belong to a unique class of histone deacetylases that is plant specific. Previously, we have demonstrated that one of the members, AtHD2A, can mediate transcriptional repression when targeted to the promoter of a reporter gene (Plant J. 22,19-27). Here we report that AtHD2B and AtHD2C can also repress gene expression. AtHD2A and AtHD2C differ from AtHD2B and AtHD2D in the composition of their structural domains. Our data shows that both structural types play a role in the repression of gene transcription. We demonstrate that AtHD2A can mediate gene repression through interactions with transcription factors in plants. By fusing AtHD2A with the DNA-binding domain of the plant transcriptional factor Pti4, the expression of a GCC box-containing reporter gene was repressed. We also demonstrated repression of a GUS gene with GAL4 enhancers using transgenic plants that expressed a GAL4/AtHD2A fusion gene. Furthermore, the expression of the GAL4/AtHD2A protein using the seed-specific napin promoter (NAP2) and the constitutive tCUP promoter demonstrated that repression of transgenes could be achieved in a tissue specific or unrestricted manner. Targeting of HD2 proteins to specific promoters using transcription factor DNA binding domains may therefore provide a new technology for silencing target genes and pathways in plants as well as for assessing the function of unknown transcription factors.

95 Right-handed helical growth in Arabidopsis plants expressing altered α-tubulin genes

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The recessive *spiral1* and *spiral2* mutants show right-handed helical growth in roots and etiolated hypocotyls. *spiral2* mutants additionally display right-handed twisting in petioles and petals. In elongating root epidermal cells of *spiral1* mutant, cortical microtubules (CMTs) were arranged in left-handed helical arrays. In contrast, the semi-dominant *lefty1* and *lefty2* mutants show opposite left-handed helical growth in these organs, and are caused by dominant negative mutations in α-tubulin genes. The CMTs in *lefty* mutants are arranged in right-handed helical arrays, and are more sensitive to microtubule-specific drugs than wild-type microtubules. Therefore, reduced microtubule stability may produce right-handed helical arrays in CMTs, resulting in left-handed helical growth; however, the nature of left-handed CMT arrays remains to be determined. We noticed that, when α-tubulin 6 (TUA6) was tagged with GFP at the N-terminus and expressed under the CaMV35S promoter in transgenic Arabidopsis plants, the fusion protein was incorporated into microtubule polymer and caused right-handed helical growth phenotypes in petioles and petals similar to those found *spiral2*. In contrast, β-tubulin 6 tagged with GFP at the N-terminus was also incorporated into CMT but did not produce helical phenotypes. TUA6 tagged with HA or myc at the N-terminus generated strong phenotypes; radial expansion in roots and strong right-handed twist in petioles and petals. In addition, transgenic lines expressing HA-TUA6 had increased trichome branching. These results suggest that α-tubulin with an extra sequence at the N-terminus interferes microtubule functions, leading to right-handed helical growth. The GTP hydrolysis of β-tubulin at the microtubule polymer end is triggered by the incoming α-tubulin. The N-terminus of the α-subunit is located close to the crucial residues in α that stimulates GTP hydrolysis in β. We speculate that extra amino acids in the α N-terminus block this GTPase enhancement, resulting in longer GTP cap and more stable microtubules.

helical growth;handedness;microtubule

96 Localization and functional analyses of AtAPM1 suggests a role in vesicular cycling and targeting of membrane transporters

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Current research into polar auxin transport has highlighted the dynamic nature of the efflux carrier and has led to the identification of genes that regulate both the activity and intracellular trafficking of auxin carriers. *AtAPM1*, an *Arabidopsis* homolog of the mammalian Insulin Responsive Aminopeptidase (IRAP), was recently cloned and characterized. *APM1* is expressed in tissues that accumulate auxin, produce aglycone flavonoid inhibitors of auxin efflux, and exhibit binding to the auxin efflux inhibitor NPA. We hypothesize that *APM1* may play a key role in the asymmetric plasma membrane targeting of the PIN1 auxin efflux carrier that is analogous to that of IRAP-mediated targeting of the GLUT4 glucose transporter in mammalian tissues. Inhibitor studies of PIN and APM1 immunolocalization in mutant and WT backgrounds support this interaction. APM1 co-localizes with PIN1 on basal plasma membranes at different developmental stages in the Ws, Col, and Ler ecotypes of Arabidopsis. When not basally localized, APM1 is localized in the endomembrane system. Mutants lacking flavonoids, natural inhibitors of both auxin transport and APM1 enzymatic activity, have altered APM1 localization patterns. The auxin efflux inhibitor NPA partially restores the localization of PIN in some flavonoid deficient mutants. *APM1* is apparently an essential gene, as indicated by the lethality of homozygous null mutants. A T-DNA insertion in the *APM1* promoter of *atamp1-1* results in mRNA expression <5% of wild type levels and decreased basal PIN1 localization. Consistent with its hypothesized role in PIN trafficking, direct protein affinity assays indicate that APM1 interacts with PIN2. Yeast two-hybrid analysis indicates interaction of APM1 with other transport proteins, including a voltage gated anion channel and a nitrate transporter. Consistent with light regulation of both APM1 activity and NPA inhibition of seedling growth, the light signal transduction protein DET1 was also identified as an APM1 interactor in the yeast two-hybrid screens.

97 Is SPIKE1 a Guanine Nucleotide Exchange factor for plant Rho-family GTPases

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Arabidopsis leaf trichome development provides a simple model to gain insight into the control of cell-fate and morphogenesis. Our lab is using Arabidopsis trichome morphology mutants to better understand the relationship between signaling, the cytoskeleton, and cell shape control. Actin and microtubules play essential roles during trichome development. Immunolocalization, GFP probes directed against cytoskeletal proteins, and pharmacological experiments demonstrate that microtubules are involved in initial polarized growth, while actin has more of a maintenance function. *spike1* was isolated in a screen to identify genes that regulate cytoskeletal organization in plants. *spike1* is a seedling lethal mutant having trichome, cotyledon and leaf-shape defects. The trichome phenotype and cytoskeleton defects suggest that SPK1 regulates the microtubule cytoskeleton. SPK1 contains several conserved domains. A 400 amino acid domain near the C-terminus (DOCKER) is common to the DOCK family of proteins. DOCK family is comprised of a diverse set of adaptor proteins found in human, flies, and worms that mediate cytoskeletal re-organization in response to extracellular signals. Localized activation of Rho-family GTPases has long been hypothesized as a key component of DOCK protein function. Consistent with this idea, it has been shown recently that the DOCKER domain defines a new class of guanidine nucleotide exchange factor (GEF). Plants have a unique family of Rho-type GTPases called ROPs (Rho of Plants) that affect cytoskeletal organization, but no exchange factor has been identified. Our aim is to determine if SPIKE1 activates plant ROPs, and if so, to understand the consequences of this activity on cell morphogenesis. Preliminary studies using the yeast-two-hybrid assay suggest that the C-terminal region of SPIKE1 interacts with several ROPs in a nucleotide-sensitive manner. Further biochemical studies are underway to determine if this interaction is direct.

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98 Essential genes with unknown functions in Arabidopsis

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Many genes in Arabidopsis are currently annotated to encode proteins with unknown functions. An important goal of current genomics research is to determine what cellular functions these proteins perform. In our SeedGenes Project (www.seedgenes.org), we are studying genes that are essential for seed development. More than 20% of the genes in the evolving SeedGenes database appear to lack established protein motifs and are predicted to encode proteins with unknown functions. These genes represent a valuable subset of unknowns suitable for further study because they are known to be essential. We are taking two complementary approaches with these genes: (1) isolating other mutant alleles through reverse genetics to increase our confidence that the correct gene has been identified; and (2) identifying interacting proteins using the tandem affinity purification (TAP) tagging method.

In order to meet the first objective, we have obtained putative duplicate alleles from the Salk collection of insertion lines and established protocols for using genetic complementation tests, flanking sequence data, and PCR-based cosegregation analysis to confirm that the correct gene has been identified. These confirmation steps are particularly important with unknowns because one cannot use the predicted protein function to conclude that a logical candidate for an essential gene has been identified.

We chose TAP tagging over other methods of identifying interacting proteins because it has proved very useful in yeast and because it facilitates identification of interactions within the host organism, potentially at endogenous protein levels. Seven genes selected for initial studies have been amplified along with their promoter regions from wild-type plants. TAP-tags were added using overlap PCR and the resulting constructs were transferred with a binary vector into Arabidopsis plants, both wild type and heterozygous. An overview of results obtained to date will be presented. Recovery of TAP-tagged proteins and characterization of interacting partners by mass spectrometry will be used in the future to help decipher the cellular functions of these essential gene products.

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99 Functional analysis of multidrug resistance-like proteins involved in auxin transport in Arabidopsis

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Many aspects of stem growth are dependent on the polar movement of auxin, which is thought to be regulated at the cellular level by basal plasma membrane (PM) localization of the PIN1 auxin efflux carrier. Recently, mutations in two multidrug resistance-like (MDR) genes encoding NPA-binding p-glycoproteins (PGPs) were shown to inhibit polar auxin transport in the hypocotyls of Arabidopsis seedlings, indicating that MDR-type (p-glycoprotein) ABC transporters function in the PIN1-dependent auxin transport process. However, instead of exhibiting impaired auxin-dependent tropic bending, *mdr* mutant hypocotyls display enhanced gravitropic and phototropic responses, perhaps as a result of observed disruption of the basal localization of PIN1 proteins. Other findings support a role for AtPGP1 and AtMDR1 in regulating auxin transport. 1) Both interact with the PM-anchored FKBP-like protein Twisted Dwarf 1 (TWD1), which appears to play a role in PGP plasma membrane localization and function. *twd1* is morphologically similar to *mdr1 pgp1* double mutants and exhibits similarly reduced auxin transport profiles. The interaction between the PGPs and TWD1 is disrupted by the auxin efflux inhibitor napthylpthalamic acid (NPA). 2) The *brachytic2* (*br2*) dwarf mutant in maize, which contains a defective PGP1 ortholog, exhibits disrupted vascular tissue formation and slight root agravitropism. *br2* mutants show light-dependent, tissue specific defects in auxin transport analogous to those seen in the Arabidopsis *mdr* mutants. What is not clear, however, is the mechanism by which AtPGP1 and AtMDR1 regulate auxin transport. Although PGPs may modulate the activity of other transport proteins, such as PIN1, it is also possible that they directly transport auxin or compounds which regulate auxin transport. Auxin transport could not be demonstrated when PGPs were expressed in a heterologous yeast system, even when 9 endogenous ABC transporters were knocked out. Co-expression of PGPs and PIN proteins in other heterologous systems may be more effective.

100 BUL elaborating form in Arabidopsis

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University of Missouri-Columbia

Adult morphology in animals is patterned during embryogenesis through coordinated cell division and migration. In contrast, little of an adult plant's morphology is determined during embryogenesis. Rather, only the basic stem and root polarity of the plant is determined by embryonic cell divisions, while form is elaborated post-embryonically mainly through cell expansion and localized somatic cell divisions. Because the wall of a plant cell fixes all cells in space, cell migrations do not contribute to pattern. However, the plane of cell division and expansion, as well as number of cell divisions occurring has a dramatic impact on the overall morphology. Thus, alterations in either, or both, the number or plane of cell division and expansion can have drastic influences on plant form. In a screen for mutations that would suppress the differential growth phenotypes of an *nph4/arf7*-null mutant of Arabidopsis, we identified a dominant gain-of-function mutant with very distinct aerial tissue phenotypes. Most notably, rosette leaves are extremely convoluted and the inflorescence stems are dramatically thickened. We have named this mutant bul since its leaves resemble those of Butterhead lettuce (*Lactuca sativa*). The *bul* phenotypes appear to be conditioned by changes in both the number and size of cells in the affected tissues. We are in the process of determining the identity of the *BUL* gene and its protein product. Further study of BUL and its affects on cell division and expansion is likely to contribute significantly to our understanding of how plants elaborate form.

101 Analysis of the *CPLCH2* Locus

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University of Minnesota

In the laboratory of M. David Marks, we study the molecular mechanisms responsible for the development of trichomes. The formation of trichomes at regular intervals across the leaf surface results from the interplay of proteins which both positively and negatively regulate trichome initiation. Our current work focuses on a member of the *CAPRICE-LIKE* gene family, the *CPLCH2* gene. The *CPLCH2* locus consists of duplicated single-repeat myb open reading frames located approximately 2,600 base pairs apart. The annotated Arabidopsis genomic DNA sequence predicts the presence of the downstream myb *(Myb2)*, but fails to recognize the upstream myb *(Myb1)*. The two open reading frames were discovered when RT-PCR was used to clone the predicted myb *(Myb2)* sequence. Sequencing of RT-PCR products revealed two distinct DNA species. The smaller product encoded Myb2, and the larger product consisted of two overlapping, out-of-frame myb-encoding sequences. BLAST comparisons with Arabidopsis genomic DNA confirmed that the unusual larger transcript appears to be real as opposed to a technical artifact.

A second Myb1/Myb2 combination transcript was obtained from another RNA source; like the initial combination transcript, this transcript followed normal GT/AG intron splicing patterns and confirmed that the two mybs are indeed out-of-frame. When RT-PCR was performed using forward primers upstream of both mybs, results indicated that both upstream sequences are transcribed. Subsequent 5' RACE experiments confirmed that separate promoters lie upstream of *Myb1* and *Myb2* and further showed that transcription can begin in intron 1 of *Myb1*. 3' RACE experiments revealed that multiple different polyadenylated transcripts exist for both *Myb1* and *Myb2*. Transgenic plants which overexpress a *Myb2::GFP* fusion construct using a *35S CaMV* promoter show loss of leaf trichomes, and we are currently studying the phenotype of plants homozygous for T-DNA inserts within *CPLCH2 Myb2*. Future experiments will include real-time RT-PCR for quantification of the myb transcripts in different plant tissues at various developmental stages, and placement of the *GUS* reporter gene under the control of the presumptive *Myb1* and *Myb2* promoter regions to determine temporospatial expression patterns of the two mybs.

102 Molecular levels of plastid division proteins FtsZ1 and FtsZ2 and analysis of their interactions

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Michigan State University

FtsZ forms the cytoskeletal framework of the bacterial cell division apparatus through dynamic GTP-dependent self-assembly, forming a ring-like structure at the division site that constricts with cell septation. Although one *FtsZ* gene is sufficient for cytokinesis in most prokaryotes, two homologous but distinct forms of FtsZ, FtsZ1 and FtsZ2, are required for chloroplast division in plants and both proteins colocalize to a ring at the plastid division site. To gain insight into the functional relationship between FtsZ1 and FtsZ2, we have used a series of immunological methods to investigate their molecular levels, stoichiometric relationships and interactions in chloroplasts of *Arabidopsis thaliana*. Antipeptide antibodies were generated to specifically recognize each of the three FtsZ gene products present in Arabidopsis, AtFtsZ1-1, AtFtsZ2-1, and AtFtsZ2-2. Quantitative immunoblotting demonstrated that the average chloroplast of a 3-wk-old Arabidopsis plant contains 101,200 (+/-) 5000 molecules of total FtsZ. Based on measurements of chloroplast circumferences at this stage of development and structural data available for bacterial FtsZ, we estimate that, if assembled end-to-end, there is enough FtsZ to encircle the chloroplast 20 times. Intriguingly, the amount of FtsZ in the chloroplast relative to its circumference is almost identical to that estimated for actively dividing E. coli cells. Further, the FtsZ concentration in the chloroplast exceeds that required for full GTPase activity and cooperative assembly of *E. coli* FtsZ *in vitro*. Although total chloroplast FtsZ levels were 10-fold lower in 7-wk-old than in 3-wk-old plants, the molar ratio between FtsZ1 and FtsZ2 remained constant at 1:2 throughout leaf development, suggesting this stoichiometry may be critical for FtsZ ring formation in the chloroplast. Consistent with the colocalization of FtsZ1 and FtsZ2 at the division site, coimmunoprecipitation experiments on *in vitro* translated Arabidopsis FtsZ proteins imported into isolated pea chloroplasts, as well as 2-D native-SDS-PAGE separations of proteins present in chloroplast lysates, provide strong evidence that FtsZ1 and FtsZ2 interact directly both *in vitro* and *in vivo* as components of a functional complex.

103 rol mutations suppress aberrant cell wall development of lrx1 mutants

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The cell wall is the primary determinant of the shape of an individual cell and ultimately of the whole plant. However, the knowledge on the structure of the cell wall and the regulation of cell wall development is still limited. We have identified in Arabidopsis a family of eleven *LRX* genes coding for extracellular proteins consisting of a N-terminal LRR domain and a structural extensin moiety at the C-terminus. The presence of a LRR domain suggests a regulatory role of these proteins in the process of cell wall development. LRX1 is specifically expressed in root hairs and localizes to the extracellular matrix. Root hairs of *lrx1* knock-out plants are short, form bulbous structures at their basis and frequently disrupt. Ultrastructural analysis has revealed severe deficiencies in the structure of the extracellular matrix, suggesting that LRX1 is involved in the regulation of cell wall development. *LRX2*, a paralog of *LRX1*, is also expressed in root hairs and synergistically interacts with *LRX1*. While *lrx2* knock-out mutants do not display aberrant root hairs, *lrx1/lrx2* double mutants show an enhanced *lrx1* phenotype. To better understand the process LRX1 and LRX2 are involved in, we have performed an EMS mutagenesis on the *lrx1* mutant and have isolated revertant lines that display wild-type root hairs. Several recessive and dominant *rol* (repressors of *lrx1*) mutants were found in this screen. The *rol* mutations are potentially affected in components of the LRX1/LRX2-dependent cell wall development. The repressive activity of the majority of the *rol* mutants depends on the presence of an intact *LRX2*, confirming that LRX1 and LRX2 have similar functions during cell wall development. Mapping of the *rol* loci is currently in progress.

104 *rol* mutations suppress aberrant cell wall development of *lrx1* mutants

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University of Zurich

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105 Mutation In sec61a Causes Severe Developmental Defects in Arabidopsis

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Sec61 acts as the gateway for secreted protein targeting in the cell, signal recognition of targeted proteins, and the insertion of the trans-membrane domain into the lipid bilayer. These all take place on the endoplasmic reticulum at the site of the Sec61 pore complex. This complex comprises three subunits of the Sec61 protein, which provide a connection between the ER lumen and the cytoplasm. Various other proteins involved in signal recognition and processing are also associated with this complex. Experiments in yeast indicated that mutations in Sec61α, and Sec61γ result in cell death, and mutations in Sec61β significantly reduce cell growth, demonstrating the importance of these genes in basic cellular function. Using map-based cloning, we identified a recessive mutation in the Sec61β gene corresponding to a 46 bp deletion. Seedlings from this mutant population develop very short roots, and make considerably more root hairs. Almost every cell in the epidermis gives rise to a root hair, so we named the mutant *rapunzel-1 (rap-1)*. We also frequently observed increases in cotyledon and leaf numbers, suggesting defects within the apical meristem. The leaves of the mutant plants were significantly smaller than those of the wild type, and the mutant produces no viable seeds. The wild type gene incorporating a 3′-translational GFP fusion complemented these mutant phenotypes, confirming that the mutation within Sec61β is responsible for the developmental defects described above. Further studies using various GFP reporter lines indicated that cells containing the mutant Sec61β gene have lost the ability to target proteins to the plasma membrane and to the vacuolar membrane. We have also identified those genes whose expression patterns were altered by the Sec61β mutation, using genome-wide long oligonucleotide microarrays.

106 Seed mucilage as a genetic target for pectin production

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Carnegie Institution

The cell wall is a defining feature of plants, however its heterogeneity and structural complexity have made it difficult to study. Upon seed imbibition in Arabidopsis, pectin is extruded from a single layer of cells on the outside of the seed coat. This pectin, or mucilage, is non-essential for viability, and compared to cell walls it is structurally simple. Therefore, Arabidopsis seed mucilage makes an excellent genetic and biochemical model system. Two mutants previously identified in a seed development screen, mucilage modified 3-1 and mum5-1 (Western et al., 2001), have seed mucilage with aberrant physical properties. Map based cloning of mum3-1 and mum5-1 is underway. In the mum5-1 mutant, a putative pectin methylesterase possesses a mutation that results in a change in a conserved amino acid. Pectin methylesterases (PMEs) are pectin modifying enzymes that affect pectin charge. In an independent screen in our laboratory, thirty-two additional mutants that have mucilage with altered physical properties were identified. These mutants, as well as mum3-1 and mum5-1, are being classified based on the biochemical properties of the mucilage. Classification criteria are sugar composition, electrophoretic mobility, and degree of methyl-esterification. The classification of mutants will aid in the selection of biologically interesting mutants. Predicted gene targets include cellular machinery necessary for pectin biosynthesis, modification, secretion, and/or the regulation of these processes. The identification of a pectin-modifying enzyme in a seed mucilage mutant validates the use of seed mucilage as a phenotypic criterion.

Western TL, Burn J, Tan W-L, Skinner DJ, Martin-McCaffrey L, Moffatt BA and Haughn GW (2001). Isolation and characterization of mutants defective in seed coat mucilage secretory cell development in Arabidopsis.Plant Physiology 127: 998-1011.

107 A comprehensive analysis and database of the Arabidopsis thaliana thylakoid proteome defined by multi-dimensional protein separation, mass spectrometry and prediction

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This poster presents a comprehensive experimental and prediction analysis of the Arabidopsis thaliana thylakoid proteome defined by multi-dimensional protein separation, mass spectrometry and prediction. The data will be deposited in a public plastid proteome database (http://cbsu.tc.cornell.edu/vanwijk/). The peripheral thylakoid proteome was collected by sequential extraction with chaotropic agents and Triton X-114 -followed by 2-phase separation-, and separation on 2-Dimensional gels. The hydrophobic integral thylakoid membrane proteome was subfractionated based on hydrophobicity, using sequential organic solvent extraction, followed by separation by RP-HPLC or 1-DE gels and identified through nano-LC-ESI-MS/MS. To maximize identification, hydrophobic proteins were cleaved into peptides prior to MS analysis by proteolysis or by cyanogen bromide or acid hydrolysis. The lumenal proteome was earlier described by us and others. Over 100 proteins were identified, including most of the known photosynthetic apparatus, numerous paralogues of known photosynthetic proteins, hydrophobic proteins with very low expression levels, such as cpSecE, and several dozens chloroplast proteins with no obvious function. To evaluate the success rate of this experimental analysis, and to facilitate future studies, a dataset of thylakoid proteins was compiled with their unique AGI locus number, using the experimental data from the literature and databases, as well as the proteins identified in this study. The stroma, lumenal (split into Sec and TAT substrates) and transmembrane plastid proteomes and their physical-chemical properties were predicted using a combination of old and new neural network predictors and filters. We comment on biological functions of the experimental and theoretical thylakoid proteome.

108 A G protein-mediated signaling network in the cytoskeletal control of cell shape in Arabidopsis

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Many cellular functions in plants (e.g., polar cell expansion, cell division, organelle movement) depend on changes in dynamics and organization of actin and/or microtubule (MT) in response to internal or external cues. An important but poorly understood question in plant cell biology is how signals regulate cytokseletal dynamics/organization. We are using Arabidopsis leaf epidermal pavement cells as a model system to investigate cellular signaling that regulates the cytoskeleton in the control of cell morphogenesis. Since they are intercalary jigsaw-shaped cells, the development of pavement cells presumably requires cell-to-cell signaling. Localized expansion in one cell must be coordinated with localized inhibition of growth in opposing sites of a neighboring cell, allowing the formation of intercalary lobes and sinuses. Localized expansion requires cortical actin, whereas localized growth inhibition involves transverse cortical MTs. Using a loss-of-function approach, we have shown that two PM-localized ROP GTPases (ROP2 and ROP4) act redundantly to control shape formation in pavement cells through a coordinate regulation of both cortical actin microfilaments and microtubules. To promote lobe formation, ROP2/ROP4 are activated locally and activated ROPs promote the formation of cortical F-actin as well as suppress the formation of transverse cortical MTs in the lobe. Genetic and cell biological studies have shown that ROP2/ROP4 directly regulate two functionally distinct target proteins, termed RICs (ROP-interactive CRIB-containing proteins). FRET analysis shows that both RICs specifically interact with the activated form of ROP2 in vivo. One RIC is associated with cortical MTs and promotes the formation of transverse MTs in the neck. Activated ROP2 inactivates this RIC by sequestering it from cortical MTs. Another RIC is activated by ROPs and promotes the formation of cortical actin. These observations indicate that the ROP GTPase switch orchestrates at least two downstream pathways to coordinate the actin and microtubule cytoskeleton in its spatial control of cell expansion in plants.

109 Biochemical and genetic analyses of the Arabidopsis fimbrin gene family

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Higher order actin arrays are present in many different types of plant cell, but are especially prominent in pollen tubes and root hairs. The formation of higher actin structures such as the bristle of *Drosophila* or the microvillus of mammalian intestine requires the coordinated efforts of at least two actin_crosslinking or bundling proteins. Although the function or the formation of these actin structures in plants is largely unknown, we reported previously that ATFIM1 from *Arabidopsis* can crosslink actin filaments to form supramolecular structures *in vitro* and *in planta* (Kovar et al. 2000). Although molecular evidence confirms the presence of other actin-crosslinking proteins such as villin, EF-1a, and annexin, to date, ATFIM1 is the only protein demonstrated to be a bona fide actin-crosslinking or bundling protein in *Arabidopsis*. *ATFIM1* is a member of the fimbrin gene family that includes four other members. We are using an integrated approach of biochemical and reverse genetic tools to examine the function of each member of the fimbrin gene family and its roles in modulating higher order actin structures. Our preliminary biochemical data suggest that ATFIM2 binds with high affinity, and can bundle actin filaments in a calcium-independent fashion. Additionally, we are performing a detailed phenotypic analysis of T-DNA mutants for each of the five fimbrin genes.

110 Production and Initial Usage of an *Arabidopsis* Promoter Microarray

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To analyze the transcription factor-promoter interaction in Arabidopsis, a genome scale pilot promoter microarray has been produced. An integrated platform for promoter sequence extraction and designing of primers for PCR amplification of promoter regions of all annotated genes in a genome has been developed. This platform consists of several PERL modules and a primer-design program *Primer3*. A web-interfaced primer retrieving program was used to obtain up to ten primer pairs with proper ranking for each gene. We have selected 3,800 pairs of primers for promoters of 5 specific gene sets, including transcription factors, ubiquitin proteasome degradation related proteins, and kinases. A success rate of >95% was achieved for PCR amplification of promoter fragments from total genomic DNA. Those PCR products were purified and used to print our first generation of Arabidopsis promoter microarray.

In its first application, this promoter microarray was used to study the *in vitro* binding of a transcription factor HY5 to promoters. A set of promoter fragments exhibited consistent strong interaction with HY5 protein in vitro. Based on those binding affinity, conserved motifs among these possible promoter targets were identified by a Gibbs sampling approach. Our study showed that the combination of promoter microarray and in vitro protein binding experiment can be an efficient method for identification of transcription factor binding sites in a large scale. More importantly, the availability of the promoter microarray, together with the ChIP (Chromatin immunoprecipitation), should provide required tools to analyze *in vivo* protein-DNA interaction at genomic scale.

111 An inhibitor of ubiquitin-dependent proteolysis induces cell death in Arabidopsis

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Modification of proteins by covalent addition of the small protein modifier ubiquitin is essential for many regulatory processes. Attachment of a multi-ubiquitin chain linked via Lys48 of ubiquitin targets substrate proteins for degradation by the proteasome. We have lowered the capacity of the ubiquitin proteasome pathway by expression of ubR48, a ubiquitin variant that cannot form multi-ubiquitin chains linked via Lys48 of ubiquitin. *Arabidopsis* plants expressing the inhibitor show a marked tendency to initiate cell death. These cell death events are accompanied by production of reactive oxygen intermediates. Elimination of SA by expression of the *nahG* gene does not decrease cell death. In order to dissect the process, a line containing an inducible construct for ubR48 expression was subjected to EMS mutagenesis. Mutants that survive an otherwise lethal expression level of the inhibitor ubR48 are being characterized.

112 GFP-fluorescence profiling of plant cell division related proteins

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Plant cytokinesis is more complex than in animals due to the presence of a rigid external cellulose wall. Testimony to the difficulties the cell wall imposes on the division process are the preprophase band (PPB) and the phragmoplast, two cytoskeletal structures that are necessary to assure adequate positioning and assembly of a new separating cell wall. Unlike other eucaryotes, the position of the division plane is established by the PPB during the G2-phase. The PPB disassembles upon entry into M-phase and leaves behind a hitherto unidentified positional cue that helps to guide the expansion of the cell plate toward the mother wall. The phragmoplast, which emerges at the cell center, serves as a scaffold to build the cell plate by directing Golgi derived vesicles containing callose synthesis complexes to the equatorial plane. In order to build a comprehensive list of the components that are associated to these structures, we have explored the Arabidopsis genome for potential cytoskeleton and division related genes and localized the respective GFP-fusion proteins in dividing tobacco BY-2 cell suspension culture cells. A collection of more than 100 ORF?s were identified that included proteins with microtubule or actin binding properties, sequences with homology to S. pombe, S. cerevisiae and animal genes previously implicated in cytokinesis, and 25 Arabidopsis ORF?s of unknown function that are the presumptive orthologues of tobacco cDNA tags specifically expressed during S-, G2-, or M-phase. The fluorescence profiles of BY-2 cultures transformed with 75 fusion constructs with GFP placed at the C-terminal end, were determined. The analysis resulted in 56 specific intracellular localization patterns and allowed to discriminate different subcellular localizations of proteins belonging to a single, conserved protein family. Three fusion proteins associated with the PPB and 12 proteins distinctively labeled separate regions of the phragmoplast. Drug analysis distinguished the phragmoplast associated proteins in vesicular and microtubule binding proteins. Furthermore, time-lapse confocal microscopy suggested that a protein of unknown function is implicated in the directional expansion of the cell plate and its insertion into the mother wall.

113 Ethylene Inhibits Trichome Formation on Stems and Branching on Leaves

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Cellular differentiation represents a critical aspect of organismal development. In plants, trichome (leaf hair) development provides a convenient model system for studying cellular differentiation. Previously, gibberellins have been shown to exert a relatively small, positive effect on trichome formation. Results presented here indicate that ethylene can exert a dramatic negative effect on trichome formation and branching. The *constitutive triple response1* (*ctr1*) mutant of Arabidopsis displays a constitutive response to ethylene. Two independent *ctr1* mutants, *ctr1-1* and *ctr1-12/sis1-1*, were found to have significantly decreased numbers of stem trichomes. The effects of mutations in *CTR1* can be phenocopied by supplying wild-type plants with exogenous 1-aminocyclopropane-1-carboxylic acid (ACC), an ethylene biosynthetic precursor. Growth in the presence of 50 uM ACC results in the almost complete elimination of stem trichomes. The trichomes produced on Arabidopsis leaves are branched, unlike those produced on stems. The *ctr1-1* and *ctr1-12/sis1-1* mutations exert no dramatic effects on leaf trichome number or branching. Similarly, growth of wild-type plants on media containing 50 uM ACC also has no striking effect on leaf trichome number. However, growth on 50 uM ACC results in the production of unbranched rather than branched trichomes on both rosette and cauline leaves. In addition, electron micrographs reveal that leaf trichomes from plants grown on 50 uM ACC have a distorted cell surface, with spiral ridges running the length of the trichomes.

114 Expression analysis of members of the endo-polygalacturonase gene family from *Arabidopsis thaliana*.

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Cell separation is a critical developmental process that takes place throughout the life cycle of a plant. It contributes to the emergence of the root from the germinating seed, the expansion of the leaf, the formation of the stomatal aperture, the growth of the stem, the development of pollen, the dehiscence of anthers and pods, and the shedding of organs at the end of their natural life. One of the key enzymes involved in the degradation of cell wall is the hydrolytic enzyme Polygalacturonase (PG). With the publication of the entire *Arabidopsis* genome, it is now possible to identify 69 putative members of the PG gene family and by sequence comparison place these into a Phylogenetic tree from which four distinct clades can be identified. By comparing these sequences with PGs reported from other plant species it is possible to perform a more detailed classification of this family of genes. By fusing the promoter of different PGs to the reporter gene GUS, and confirmation by RT-PCR, we have analysed the spatial and temporal expression pattern of five members of the Arabidopsis gene family. Our results show that each family member is expressed at a discrete site where cell separation would be anticipated to take place and that whilst four of these PG genes have close sequence homology and similar gene structure their patterns of expression do not overlap.

115 Localization of an ascorbate-reducible cytochrome b561 at the tonoplast

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Through its function in modulating reactive oxygen species levels, and as a cofactor to numerous metabolic enzymes, ascorbate plays a key role in the control of plant growth, development and defense responses. Despite the importance of vitamin C, a class of membrane proteins, cytochromes b561 (cyt b561), that play a key role in cellular ascorbate metabolism has only recently been identified in plants. Cyts b561 catalyze ascorbate-mediated trans-membrane electron transport, thereby contributing to redox status regulation in plant cells.

Four cyt b561 isoforms (AtCB 1- 4) have been identified in Arabidopsis. Little is know, however, on the physiological function and tissue and subcellular distribution of these proteins. Biochemical evidence has demonstrated the presence of an ascorbate-reducible cyt b561 in Arabidopsis plasma membranes. It remains unclear, however, which of the isoforms is represented by this activity.

Using promoter::GUS transformants we will demonstrate that cyts b561 are primarily expressed in Arabidopsis roots and that expression patterns are specific for each isoform.

To address the subcellular localization of cyts b561, antibodies were generated against C-terminal peptides. The antibodies against one of the isoforms, AtCB1, specifically recognize the recombinant protein in yeast, and cross react with a partially purified ascorbate-reducible cyt b from Arabidopsis leaves. Membranes were fractionated using sucrose density gradient centrifugation. The cross reaction of the AtCB1-antibodies correlates closely with the distribution of the tonoplast marker, but not with any of the other markers. In addition, Western blots with highly purified tonoplast and plasma membrane fractions demonstrate a strong enrichment of AtCB1 in de vacuolar membrane.

Measurements of reduced-minus-oxidized absorbance spectra in sucrose gradient fractions and purified membranes further supports the presence of an ascorbate-reducible cyt b in the tonoplast.

The localization of an ascorbate-reducible cyt b at the plant tonoplast strongly suggests that the Arabidopsis cyt b561 isoforms are located in distinct subcellular compartments and likely support various ascorbate-related physiological processes. This localization specifically opens new perspectives on the regulation of the vacuolar redox status.

116 Changes in Expression of Autophagy Genes in Arabidopsis thaliana during Nutrient Stress in Culture

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Autophagy is a regulated recycling process that enables organisms to survive during starvation through the breakdown of cellular constituents. Though autophagy is found in plants, little is known about what induces autophagy or what molecular components are involved. A nitrogen-deficient cell liquid-culture system was developed in order to determine the environmental conditions that will trigger autophagy in plants. We have used this system to identify a molecular marker for autophagy using RT-PCR. Expression of the Arabidopsis homologs to the yeast genes *APG3*, *APG6*, and *APG9* increases over time during nitrogen deficiency. We have also identified a series of knockout mutants with putative defects in predicted autophagy genes in Arabidopsis. Each mutant line has been subjected to nutrient deficiency using the cell culture system we developed. The molecular analysis of these mutant cell lines will provide additional insight into the process of autophagy in plants.

117 Deletion analysis of promoters that drive expression in tracheary elements

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A functional water-conducting system is required to sustain plant growth and development. In the secondary tissues of vascular plants, the differentiation of water-conducting tracheary elements is the result of the orchestrated regulation of vascular cambium formation, initiation of xylem differentiation, cell elongation, secondary wall thickening, programmed cell death (PCD) and cellular autolysis. Tracheary element PCD and autolysis are probably dependent on the activity of hydrolytic enzymes such as xylem cysteine protease 1 (XCP1) and xylem serine protease 1 (XSP1). Due to the scarcity of information on molecular signals triggering the expression of genes involved in developmentally programmed cell death and the lack of mutants that affect the PCD and autolytic phases of tracheary element differentiation, XCP1 and XSP1 may be valuable models for understanding the regulation of these final phases of tracheary element differentiation. The 0.6 kb and 1.82 kb upstream regions of *XCP1* and *XSP1*, respectively, drive GUS reporter gene expression in tracheary elements. Additionally, we previously showed that XCP1 localizes to vacuoles of TEs. As a follow up to these efforts, 5‘ unidirectional deletions of putative promoters for *XCP1* and *XSP1* fused to a GUS reporter gene have been used to transform Arabidopsis. Results from histochemical staining for GUS activity driven by the *XCP1* promoter deletions obtained thus far suggest that deletion of up to 223 bps from the 5‘ end of the *XCP1* promoter does not affect tracheary element-specific expression. GUS activity assays on seven deletions from the promoter for *XSP1* have revealed the existence of two regions that may be critical to maintaining normal levels of *XSP1* promoter activity and a minimal promoter (114 bp) that may be sufficient for tracheary element-specific expression of *XSP1*. The identification and characterization of elements required for expression specific to the autolytic phase of TE differentation will set the stage for experiments aimed at the identification of trans-acting factors that regulate the final events of tracheary element differentiation.

118 Toward functional genomics of *Arabidopsis* gametophytes

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With the completion of the *Arabidopsis* genome sequence, the challenge to understand the function of each gene is before us. This effort will require comprehensive genetic strategies that address the functions of large sets of genes in specific cell types. The haploid gametophytes offer a tremendous experimental system for the study of cell-cell interactions, polarity determination, and invasive cell growth, along with many other basic cellular functions. The gametophytes are structurally less complex than the diploid sporophyte yet carry out a vast array of functions and express the majority of the genome. Indeed, as much as 10-20% of the genome may be specifically expressed in pollen. Unfortunately, traditional genetic approaches are insufficient to address the functions of this set of genes because mutant alleles are not transmitted efficiently and homozygous plants are often not recovered. We have initiated a comprehensive screen for haploid-expressed genes critical for gametophyte development and function. The long-term goal of this work is to obtain mutants in each of the estimated 1000 genes that are essential for the gametophyte generation. Our approach marks mutant pollen with a histochemical reporter (LAT52:GUS) and uses the quartet mutation to track distorted segregation in pollen tetrads. Thus far, > 10,000 independent transgenic lines have been screened and thirty-two *hapless* (defective haploid cells) mutants have been identified. The majority of *hap* mutations are specifically affect pollen, a large class affects both gametophytes, and two *hap* mutants are female-specific. We have identified putative T-DNA insertion sites for several hap mutants using TAIL-PCR. These results indicate that the genes required for the gametophytes represent a diverse set of known functional categories. Importantly, we have assigned novel functions for multiple genes with previously unknown functions. This project will generate a valuable genetic resource for basic studies in cell biology and biochemistry and will enhance our understanding of unique pollen functions such as guided tip growth of the pollen tube. In addition, this screen offers the unique opportunity to understand the complement of the *Arabidopsis* genome that is required for the function of a single cell.

119 Characterisation of the *Arabidopsis* Tic Machinery

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Chloroplast function depends on efficient import of more than 90% nucleus-encoded proteins. The import of these proteins across the outer (Toc complex) and inner (Tic complex) envelope membranes is facilitated by multimeric translocation complexes. The core components of the Tic complex are proposed to be Tic110 and Tic40, as well as a stromal Hsp100 molecular chaperone, ClpC. Biochemical studies with pea chloroplasts strongly suggest that these components interact *in vitro* and with arrested translocation intermediates. We have identified and characterized four *Arabidopsis* knockout mutants for the single *atTic110* and *atTic40* genes, and for the two genes encoding chloroplast Hsp100 isoforms, *ClpC-III* and *ClpC-V*. The genes are all expressed throughout plant development. The mutants have a pale phenotype, altered chlorophyll content and ultrastructural chloroplast defects. Involvement in embryogenesis and early stages of plastid development is suggested for Tic110. We have also been investigating the import defects in detail using various precursors. Further evidences supporting an *in vivo* role of these components in chloroplast biogenesis and chloroplast protein import will be discussed.

120 *DAB5*, a secretory pathway gene, is involved in abscission in Arabidopsis

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Abscission, the shedding of organs such as leaves, floral organs, and fruits, is essential during stages of plant development, yet the physiological, cellular, and genetic processes regulating abscission remain unclear. What is evident is that abscission is an active, complex process that occurs at defined zones of distinct cells (the abscission zone) that undergo cell separation as a result of breakdown of the middle lamella and the cell wall matrix. By screening the University of Wisconsin T-DNA tagged mutant populations in Arabidopsis, we have identified mutants with delay in floral organ abscission. One of these mutants, *dab5-1* (delayed abscission 5-1), fails to shed floral organs throughout the life cycle of the plant and is regulated by a single recessive gene. Scanning electron microscopy photos of the fracture plane of the abscission zone of *dab5-1* plants indicate that the cells in this region undergo uncontrolled elongation. Using inverse and tail PCR we isolated a T-DNA insertion upstream of a gene predicted to be involved in vesicle trafficking in the secretory pathway. Northern blot analysis has shown the loss of expression of this gene in *dab5-1* plants. Southern blot analysis and PCR confirm a single T-DNA insertion co-segregating with the mutant phenotype. Molecular complementation and identification of additional alleles are underway to further confirm that this insertion is responsible for the delayed abscission phenotype. We will present the phenotypic and physiological characterization of *dab5-1* and preliminary genetic and molecular analysis.

121 Arabidopsis DISTORTED1 Encodes a Plant Actin-related Protein AtARP3

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It has been proven that the genetic approaches are very important tools for understanding how the organization of the cytoskeleton and associated proteins is regulated in response to both intrinsic and extrinsic cues. Arabidopsis trichomes are unicellular, highly polarized epidermal cells, which display distinct requirements for both microtubule and actin-filament cytoskeletons during their developmental procedures. The long-term goal of our research is to use the distorted trichome mutants to elucidate the mechanisms of cytoskeleton-dependent cell morphogenesis. We and others have identified eight loci that, when mutated, cause a distorted trichome phenotype. The distorted trichome mutants display cell swelling and twisting: the timing of which precisely coincides with the developmental transition to actin-dependent cell growth. The stage-specific defects of the distorted mutants can be phenocopied by actin filament disrupting drugs. The distorted mutant, *dis1-1*, not only displays the resistance to the actin depolymerizing drug latrunculin B, but contains disorganized actin bundles in the branches of developing trichomes compared to the wild type. Allele sequencing and western blotting experiments clearly demonstrate that defects in the AtARP3 gene cause the *dis1* phenotype. ARP3 is one of seven subunits that assemble into a complex known as the ARP2/3 complex. In animal cells the ARP2/3 complex is an important nucleator of actin filaments that drives both membrane protrusion and organelle motility. Our poster will present results using distorted trichome mutants to unravel the functions of the ARP2/3 complex in plant cells.

122 Characterization and functional analysis of the P-type aminopeptidase *AtAPP1*

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AtAPP1 is an Arabidopsis ortholog of a mammalian aminopeptidase (AP) of M24 metalloprotease family involved in interactions with the extra cellular matrix, peptide signal processing, secretion, and membrane vesicularization. AtAPP1 was originally identified as a low-affinity component of NPA-binding protein complexes and was subsequently found to exist in two isoforms with distinct activities: a cytoplasmic Ca2+-activated Mn2+ X-Pro peptidase and a membrane-associated Zn2+ metallopeptidase. Consistent with its identity as a peripheral membrane protein, APP1 has two predicted hydrophobic surface regions. APP1 co-purified an *Arabidopsis* ortholog of the furin-type $Kex2_p$ protease from microsomal fractions. Both AP-P and furin-type peptidases have been associated with defense responses in tomato. *AtAPP1* is expressed in roots, leaves (cauline and rosette), flowers and siliques and in seedlings has a peak expression at 5 days. *AtAPP1* expression is induced by both light and auxin exposure. T-DNA and EMS mutants in coding and promoter regions of *AtAPP1* have been obtained.*atapp1* phenotypes include truncated root development, delay in flowering and partial silique filling. Immunohistochemical analysis of atapp1 promoter insertion mutants suggests a role in processing of PIN1 efflux carriers on the plasma membrane. Consistent with functions in stress responses and protein processing, yeast two hybrid analysis using full length AtAPP1 as bait suggests interactions with superoxide dismutase, peroxidase, carbonyl reductase and E2-ubiquitin-conjugating enzyme. This is consistent with the APP function in mammals where it is involved in stress response mechanisms and processing of stress-related peptide hormones such as bradykinin and PYY *AtAPP1*

123 Characterization of two novel kinesins and their role in cytokinesis

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The cytoskeleton is a key player in plant cell division. Unlike animal cells, plant cells are surrounded with a rigid wall. Cytoskeletal assemblies (preprophaseband, spindle and phragmoplast) are involved in the spatial and temporal organization of the cross wall between daughter cells. The *Tangeld1 (Tan1)* gene of maize has been shown to be required for the proper orientation of cytoskeletal arrays that are involved in plant cell division. Functional analysis revealed direct binding of TAN1 to microtubules (MT) *in vitro*, which suggests that TAN1 associates with MTs in vivo. Anti-TAN1 antibodies specifically decorate cytoskeletal arrays in dividing cells, suggesting, that TAN1 functions in the orientation of these arrays during cell division. In a yeast-two-hybrid screen for TAN1 interaction-partners, C-terminal portions of two kinesins were identified. A remarkable variety of plus- and minus-end directed kinesins is thought to be involved in the redistribution of cytoskeletal arrays during the cell cycle. To test the hypothesis if ATN (the *Arabidopsis thaliana* ortholog of TAN1), like TAN1, functions to orient cytoskeletal arrays in dividing cells, by mediating their interaction with other cell components, the emphasis of our work is to analyze the function and localization of two closely related kinesins, KAT1 and KAT2 of *Arabidopsis thaliana*, which are most likely interaction-partners of ATN. KAT1 already exhibited strong interaction with ATN in yeast and the expression pattern of *ATN, KAT1* and *KAT2* are overlapping. Transcript for all three genes is present in immature flower buds, which are enriched in dividing cells. Following a reverse genetics approach we identified mutants *kat1* and *kat2* that carry T-DNA insertions. The single mutants do not exhibit obvious differences from the wildtype phenotype, but the double mutant is a dwarf. Current work focuses on the analysis of the double mutant phenotype.

124 Arabidopsis Mutant *phs1* Is Hypersensitive to Microtubule Destabilizing Drugs, and Is Defective in a Protein Phosphatase

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The left-handed helical growth mutants *lefty1* and *lefty2* are caused by dominant negative mutantion in α-tubulin genes, and are hypersensitive to microtubule inhibitors such as propyzamide, oryzalin and taxol. The root of semi-dominant mutant *phs1-1* (propyzamide hypersensitive 1) grew toward left, like *lefty* roots, when grown on a hard agar surface without drugs. Whereas *lefty* mutants are hypersensitive to taxol, a microtubule-stabilizing drug, *phs1-1* showed almost wild-type response to taxol. Cortical microtubule arrays in epidermal cells of *phs1-1* roots are more fragmented than the arrays in wild-type cells. The phenotype of double mutants between *phs1-1* and *mor1* or *spr2* mutants, which are defective in microtubule associated proteins, was additive or synergistic. The *phs1-1* locus was mapped on a north region of chromosome 5, where no microtubule-related loci were reported previously. We found a point mutation that results in an amino acid exchange in one gene which encodes a putative protein similar to a dual specificity protein phosphatase. When the *phs1-1* allele of the candidate gene was expressed in wild-type plants, transgenic plants showed left-handed helical growth and increased sensitivity to propyzamide, indicating that the mutated protein acts dominant negatively.

microtubule, microtubule destabilizing drug, helical growth

125 Intracellular Movements and Myosins

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Plant cells cannot move around like animal cells, but they display a remarkable degree of intracellular movements, commonly referred to as "cytoplasmic streaming". These movements of organelles and cytosol are dependent on actin filaments and myosin motors. Even though all cytoplasmic streaming in plant cells is driven by the acto-myosin system, there is growing evidence that individual organelles can move independently of each other. For example, Golgi stacks are specifically accumulating around the spindle poles and in a cortical "Golgi belt" during metaphase, whereas mitochondria and plastids do not show this behavior. The mechanisms that mediate such organelle-specific movements are currently unknown. We speculate that the motor proteins that drive organellar motions, i.e. the myosins, are prime candidates for regulation of this process. Plant myosins share the general characteristics of all myosins, but fall into two distinct classes (VIII and XI), which are not found in other organisms. The *Arabidopsis* genome encodes 4 class VIII myosins and 13 class XI myosins. Class XI myosins are most likely involved in organelle transport, but very little is known about the specific function of individual genes in this family. We will provide an update on our ongoing efforts to characterize this gene family by cytological and genetic approaches.

126 Genetic analysis of a suppressor mutant of *zig/sgr4* defective in *AtVTI11*

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To elucidate the molecular mechanism of the shoot gravitropism, we have isolated many *shoot-gravitropism(sgr)* mutants in *Arabidopsis*. The *zigzag (zig/sgr4)* mutant exhibited abnormal gravitropism in both infloresence stems and hypocotyls. Additionally, the stems elongated in a zigzag fashion. The *ZIG* gene encodes a SNARE, AtVTI11 that is homologous to yeast Vti1p involved in vesicle transport to vacuoles. To understand how vesicle transport to vacuoles are involved in morphogenesis and gravitropism, we have isolated some suppressor mutants of *zig-1* from EMS-treated *zig-1* seeds. A dominant mutation *zig suppressor 1(zip1)* could almost completely suppress the defects of *zig-1* mutant in both morphogenesis and gravitropism. Based on the map position, we found a point mutation in the *AtVTI12* gene. Whereas AtVTI12 is one of homolog of ZIG/AtVTI11, they are, at least in part, functionally different because a *vti12* single homozygous mutant did not exhibit remarkable phenotype. On the basis of results of biochemical and cytological analyses, we will discuss the mechanism of *zig-1* phenotype suppression by zip1-type AtVTI12.

127 Expression Patterns of Cellulose Synthase-Like Gene Family Members

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The cellulose synthase-like (CSL) proteins consist of six families of 29 *Arabidopsis thaliana* transmembrane proteins thought to be processive polysaccharide β-glycosyltransferases. These proteins exhibit sequence similarity to known cellulose synthase proteins and may participate in the synthesis of cell wall polysaccharides, mucilage or the glycosyl residues found on AGP's. In order to elucidate the functions of members of this large family, the expression of each member was studied. Promoter regions from selected genes were cloned in front of a β-glucuronidase (GUS) gene and expressed in plants. In addition, microarray expression data was gathered from various tissue-specific preparations and mined for cellulose synthase-like (CSL) transcript expression activity. Data obtained from GUS-expression and microarray mining will reveal cell-specific, tissue-specific, and temporal-specific expression patterns which are necessary in assigning enzymatic and biological functions to the CSL gene family members.

128 Oryzalin Hypersensitive Mutants of Arabidopsis

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It has long been known that there is a connection between the cell wall and the microtubule cytoskeleton. Cellulose is deposited perpendicular to the direction of cellular expansion, directionally reinforcing the cell wall and directing turgor driven expansion. Disorganization of cortical microtubules with microtubule destabilizing drugs causes disorganized cellulose deposition and isometric cellular swelling. Cortical microtubules are thought to guide cellulose deposition since there is a correlation between the orientation of cortical microtubules and cellulose microfibrils. In order to find the components regulating cortical microtubule dynamics and cellulose deposition, I have isolated Arabidopsis mutants that are hypersensitive to the microtubule-destabilizing drug oryzalin. From a large-scale screen, over 130 mutant lines have been recovered and are being characterized. Cortical microtubule organization of several mutant lines has been examined with immunofluorescence microscopy and organization is clearly aberrant. We are currently focusing on a small group of mutants that are sensitive to both oryzalin and the cellulose synthase inhibitor isoxaben. The cell walls of these mutants are being examined with FESEM to visualize cellulose deposition patterns and with chemical techniques to examine the composition of these mutant plants.

129 Ab Initio Structural and Functional Characterization of Proteins Required For Meiotic Events in *Arabidopsis thaliana*

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Meiosis is an important reproductive process in plants. Attempts were made in order to elucidate the hypothetical three-dimensional structure of many proteins and their localizations encoded by the *Arabidopsis thaliana* meiotic genome. By comparative structural determination for 36 for *Arabidopsis thaliana* meiotic proteins, to disseminate and extract useful information. Structural homology to other known structures and motifs was carried out by structural proteomics.Quantification of all the proteins expressed in a cell, Knowledge of the minimum gene and protein set is required for (meiotic) plant cell division. What genes and proteins are required for meiotic cell division? What is the structure and function of these meiotic specific genes and proteins. It would be interesting to built up the simulated recipe of genes, proteins and all related events which are required for Meiosis using modern Bioinformatics, computational tools and thus goal is towards virtual cell division control. Experimental validation of these hypothetical proteins in Arabidopsis thaliana is required by using Proteomic tools like protein arrays, basic cytological and Molecular techniques to be performed in order to cross check these in silico results.

130 Protein regulators of AtCDC48 function: Membrane association, hexamer formation and plant growth

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We have taken a biochemical approach of affinity chromatography and MALDI-TOF mass spectrometry to identify possible protein regulators of AtCDC48 function during cell division. Two previously uncharacterized candidates, p28 and p52, have been identified. We show that p28 is a soluble protein that co-fractionates with non-hexameric AtCDC48 and physically interacts with AtCDC48 in vitro and in vivo. p52 is peripheral membrane protein that interacts with AtCDC48 in vitro. p52 co-fractionates with membrane-associated AtCDC48 but not with the cytosolic complex thus suggesting a role in membrane-association of AtCDC48. Two null insertion mutant alleles of p28 have been identified and show no apparent morphological abnormalities, however, data indicates that mutant plants have an accelerated growth rate. We provide evidence that p28 elicits it negative effect on growth by promoting the disassembly of AtCDC48 hexameric complexes, thereby inactivating and preventing AtCDC48 function in vivo. These data strongly support a role for AtCDC48 and p28 function in plant growth and development.

131 Characterization of a delayed floral organ abscission mutant dab4-1 that also has strong apical dominance, delayed meristem arrest, and disrupted anther dehiscence.

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Abscission can be defined as the loss of an organ from the main body of the plant including the shedding of organs such as leaves, floral organs and fruits. Rather than cell death, abscission is an active process occurring during normal plant development or in response to environmental signals. In Arabidopsis leaves do not normally abscise; however, floral organs abscise in a developmentally regulated sequence. Our model of abscission outlines four basic steps of development (ontogeny of abscission zone, dissolution of the middle lamella, cell wall loosening and cell elongation, and cell separation). dab4-1 was selected for delayed floral organ abscission by screening the Wisconsin T-DNA collection and appears to disrupt the initial dissolution of the middle lamella and cell wall loosening. In addition to delaying floral organ abscission, dab4-1 is functionally male sterile due to a failure of pollen dehiscence. dab4-1 also displays strong apical dominance, reduced lateral root development and delayed meristem arrest. The pollen from dab4-1 fails to dehisce but will germinate in the anther and grow down the style if anthers are manually dehisced. dab4-1 also continues to grow almost twice as long as wild type and more than 2.5 times taller. In addition, dab4-1 produces up to 350 flowers, almost five times the average number of flowers on the primary inflorescence. Genetic analysis indicates that a single recessive gene is responsible for this phenotype. The phenotype co-segregates with kanamycin resistance indicating the mutant is tagged or closely linked to a T-DNA insertion. We will present the phenotypic and physiological characterization of this mutant and preliminary genetic and molecular analysis.

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132 Controlling cell size through cell cycle regulators in Arabidopsis

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Higher plant growth and development depends on cell division in meristems, and the expansion and differentiation of descendant cells. Cells that are competent to divide, pass a number of key transitions controlling their entry into S-phase and mitotic phase of the cell cycle; these transitions are regulated by cyclin dependent kinases (CDKs). In fission yeast, two regulatory cell cycle genes encode enzymes that compete for the same phosphorylation site on the CDK at the G2/M transition and play important roles in regulating cell size at division. These are, a protein phosphatase encoded by *cdc25*, and a protein kinase encoded by *wee1*. We have recently isolated the *Arabidopsis* homologue to *wee1* (*AtWEE1*) and shown that its expression was confined to actively dividing tissues. Over expression of this gene in fission yeast resulted in very elongated cells and its expression in tobacco suspension cultures also had a dramatic effect both on cell size and progression through the cell cycle. However, until very recently there was no clear homologue to *cdc25* in plants. Thus, we have expressed the fission yeast *cdc25* (*Spcdc25*) in *Arabidopsis* and shown a stimulation of lateral root production and a decrease in mitotic cell size. Expression of this gene in tobacco TBY2 cells also results in a reduced mitotic cell size, with a consequent change in the plane of cell division and a close link with cytokinin signalling. Using yeast two hybrid screening to search for plant proteins interacting with the Spcdc25, resulted in the isolation of three 14-3-3 proteins, one of which (GF14 omega) showed elevated expression in dividing tissues. Furthermore GF14 omega was able to rescue the DNA damage and replication checkpoints in the fission yeast checkpoint mutant, *rad24-*. Very recently we have also identified a putative *Arabidopsis cdc25*-like homologue and shown that its expression in fission yeast has the expected hall-mark effect of a Cdc25 phosphatase by reducing mitotic cell length.

133 Functional analysis of SNAREs in the Golgi/endosomal system of Arabidopsis

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The secretory system is essential to the proper function of all eukaryotic cells. Exchange of cargo (i.e.: proteins, lipids, carbohydrates, etc.) between the different compartments of the secretory system occurs using transport vesicles that bud from a donor organelle, traverse the cytoplasm and then fuse with a target membrane. As with all eukaryotes, plants face the difficult task of directing cargo to many different destinations while using a common set of endomembrane compartments. Perhaps the most complicated point in the secretory system occurs within the Golgi and endosomal compartments. This is the cross-roads of the secretory system where cargo from as many as five different pathways converge on a common set of organelles, yet are sorted to their destination without mixing. Clearly, a complex and specific vesicle trafficking machinery is required, and one aspect of this machinery is a class of integral membrane proteins called SNAREs. Using the model plant Arabidopsis, my laboratory has identified five distinct complexes involved in Golgi-to-endosomal vesicle trafficking. Genetic evidence indicates that some members of these complexes are essential, whereas others have functional redundancy with gene family members or other SNAREs. Biochemical identification and component analysis of complexes, functional analysis of individual mutants by molecular and reverse genetics, and cell biological analysis of SNARE localization and dynamics will be presented.

134 Analysis of two monosaccharide transporter-like gene clusters

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The 14 AtSTP genes encode plasma membrane localized monosaccharide transporters and catalyze the uptake of hexoses and pentoses with different specificities in various organs and tissues of Arabidopsis. As the first functionally characterized gene cluster, which is part of an even larger superfamily of 53 genes, the AtSTPs gave the name to this larger gene family, which is called the monosaccharide transporter-like (MTL) gene family. The residual 39 genes of the MTL-gene family fall into 6 clusters of genes with relatively high sequence similarities. One of these clusters encodes proteins of the chloroplast membrane that may transport the transport of monosaccharides into this organell. Nothing is known about the physiological function, the tissue specific expression and the subcellular localization of the genes and proteins of the remaining 5 gene clusters. The genes of one cluster show reasonable sequence similarity to transporters for the linear polyols sorbitol and mannitol, which heve recently been identified in celery and common plantain, two plants that transport mannitol ar sorbitol within their vascular tissue. The genes of a second cluster show similarity to transporters described to be involved in the transport of inositol in the salt tollerant plant Mesembryanthemum crystallinum. In our studies we addressed the question after the function of these two gene clusters in Arabidopsis, which does neither transport linear polyols inits phloem and which is certainly not a slat tollerant plant. For our analyses we generated promoter::GUS and promoter::GFP plants, expressed the cDNAs in yeast and determined the subcellular localization of the encoded proteins using immunohistochemical techniques and transient expression systems. First results will be presented.

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135 ADL1 Proteins: Linking the Actin Cytoskeleton to Membrane Dynamics?

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The actin cytoskeleton plays critical roles in endocytosis and recycling of plasma membrane proteins in plant cells. Previously, we have shown that the ADL1 (Arabidopsis Dynamin-Like protein 1) protein family is required for membrane recovery from the cell plate and plasma membrane. The dependence of plasma membrane trafficking on the actin cytoskeleton suggested that ADL1 protein function might be associated with actin cytoskeleton dynamics. To investigate the relationship between ADL1 proteins and the actin cytoskeleton, we have examined the effect of actin-cytoskeleton antagonists and vesicle-trafficking inhibitors on subcellular localization of ADL1A and ADL1C.

Polar localization of ADL1A-mGFP and ALD1C in root cells and in root hairs, respectively, is disrupted by Latrunculin B (LatB). Consistent with this we have found by subcellular fractionation that membrane associated ADL1A and ADL1C is released into a cytosolic fraction upon treatment of isolated protoplasts with LatB. In addition, ADL1A-mGFP5 and ADL1C were associated with unidentified cytoplasmic structures after inhibiting secretion with brefeldin A (BFA). These preliminary experiments suggest that ADL1 localization is dynamic and may be dependent on the actin cytoskeleton.

136 Cycling Dof Factors Interact with Clock-Associated F-box Proteins

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The ZTL gene family encodes three F-box proteins which have been shown to play a role in the circadian clock of Arabidopsis. Members of this gene family (ZTL, LKP2, and FKF1) possess a unique structure containing a PAS domain at their N-terminus, an F-box motif, and 6 kelch repeats at their C-terminus. A protein:protein interaction screen was performed in yeast to identify targets of LKP2 and 3 Dof class transcription factors were identified. All 3 of these interacting Dofs are regulated by the circadian clock and they were named CDF for Cycling Dof Factors. Transgenic plants over-expressing all 3 CDFs were analyzed for phenotypes associated with the circadian clock. Over-expression of all 3 CDFs resulted in long hypocotyl phenotypes while only 1 caused arrhythmic CAB gene expression indicating a role for this Dof factor in the circadian clock. Our data suggests key and differential roles for CDFs in the Arabidopsis circadian system.

137 HOBBIT links the plant cell cycle to progression of cell differentiation

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In plant meristems, dividing cells interpret positional information and translate it into patterned cell differentiation. Recently cloning, the *HOBBIT* gene showed that it encodes a homolog of the CDC27 subunit of the anaphase promoting complex. hobbit mutants display a pleiotropic phenotype, show a reduction of the DR5-GUS auxin reporter and accumulat the AXR3/IAA17. However we still do not know whether HOBBIT affects cell differentiation by regulating the cell cycle progression in the meristem or by restricting the response to differentiation cues to dividing cells. A C-treminal GUS and GFP tagged *HOBBIT* expressed under its own promoter was still able to rescue *hobbit* mutant. HOBBIT fusion protein is nuclear localized only in dividing cells. To further studies the role of HOBBIT in the post embryonic meristem, we developed a technique based on the Cre/lox recombination system to create *hobbit* mutant sector in a phenotypicaly wild type plant. Post embryonic loss-of-*HOBBIT*-function induces cell differentiation of the meristem. GUS marker gene and expression of auxin-related proteins in postembryonic *hobbit* mutant sector will be presented. These results suggest a role of HOBBIT in control of the cell division/differentiation balance by regulating cell cycle progression in the meristem.

138 Microtubule Motility by Polymer Treadmilling in Arabidopsis Cortical Arrays

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In animal and fungal cells, interphase microtubules are nucleated from a centralized organelle that organizes the polymers into polarized radial arrays. Here we provide an analysis of single microtubule dynamics and behavior in the cortical arrays of plant cells?structured arrays that lack a centralized microtubule-organizing center and radial organization. We visualized individual microtubules by confocal imaging of new tubulin::GFP fusion proteins in Arabidopsis. New polymers initiated at the cell cortex and detached from origin sites, exhibiting dynamic behavior at both polymer ends. Growth-biased dynamic instability at the leading end and slow depolymerization at the lagging end of polymers resulted in sustained microtubule migration across the cell cortex. This hybrid form of polymer treadmilling was the principle mechanism of microtubule motility observed in the cortical array, contributing to changes in array organization through microtubule reorientation and bundling.

Shaw SL, Kamyar R, Ehrhardt DW (2003) Science. Published online April 24, 2003. http://www.sciencemag.org/cgi/content/abstract/1083529v1

139 Analysis of an abscission-associated cellulase in *Arabidopsis*

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Abscission is regulated cell separation resulting in the detachment of organs. This occurs during specific stages of development or in response to environmental signals. During the process of abscission, there is an increase in the expression of cell wall hydrolytic enzymes, including cellulases (β-1,4 endo-glucanases). We have screened the Wisconsin T-DNA collection and the SALK collection to identify knockouts in abscission-associated cellulases. We will present characterization of one of these mutants (*cel3*). The mutants did not have a delayed abscission phenotype, and SEM analysis showed no clear anatomical abnormalities in the floral organ abscission zones. In addition, petal break strength was not altered in the mutant plants. Consequently, we have also looked at other cell separation processes during development. Using RT-PCR and *in situ* hybridization we have observed differential expression of *CEL3* in various tissues and in the five stages of floral organ abscission. Our analysis indicates that this gene is involved in regulating abscission and cell separation in specific tissues; however, the lack of a clear phenotype illustrates predicted overlapping functions of the many members of this gene family. (This work was funded by USDA Grant#00-35301-9085: Genetic and Molecular Characterization of Genes that Influence Abscission and Cell Separation and NSF[subcontract with Purdue]Grant#DBI-0077719: A high through-put screen to identify cell wall biogenesis mutants of maize and Arabidopsis).

140 ARC6 is a DnaJ-like plastid division protein essential for FtsZ ring assembly

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The division mechanism of plastids evolved from the cell division machinery of their ancestors, cyanobacterial endosymbionts, and involves polymerization of the cytoskeletal tubulin-like proteins FtsZ1 and FtsZ2 into a contractile ring at mid-plastid just beneath the envelope membrane. Factors regulating FtsZ ring formation in plastids remain largely unknown. In the *Arabidopsis arc6* mutant, plastid division is blocked, resulting in cells containing only one or two grossly enlarged chloroplasts. These chloroplasts show abnormal localization of FtsZ1 and FtsZ2 and contain numerous short, disorganized FtsZ filaments. We identified the lesion in arc6 and show that the wild-type gene, *ARC6*, is closely related to a prokaryotic cell division gene unique to cyanobacteria. *ARC6* encodes a chloroplast-targeted protein spanning the inner envelope membrane that probably functions in the assembly and/or stabilization of the plastid-dividing FtsZ ring. ARC6 contains an unconventional J domain, suggesting a role for a specific Hsp70 chaperone system in plastid replication. We also analyzed FtsZ localization in transgenic plants in which plastid division was blocked by altered expression of the division site-determining factor AtMinD. Our data suggest that AtMinD acts as a negative regulator of FtsZ ring formation. Thus, factors both positively (ARC6) and negatively (MinD) affecting FtsZ ring formation play crucial roles in division of plastids.

141 Characterization of an Arabidopsis ortholog of Tangled1

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Arrays of microtubules and actin filaments are important in the spatial regulation of plant cell division. We study a mutation in maize, *tangled1*, which is required for the proper orientation of the preprophase band, spindle, and phragmoplast during cytokinesis. Although *tan1* mutant plants correctly form all cytokinetic structures they fail to guide the phragmoplast to the site of the preprophase band, resulting in a variety of aberrant cell divisions. Leaves of *tan1* mutant plants have a highly disordered cell pattern in all tissue layers. At a macroscopic level, however, *tan1* mutant plants appear nearly normal. Cloning of *Tan1* showed that it encodes a highly basic protein that is distantly related to the basic domains of ademato us polyposis coli (APC) proteins. The basic domain of APC is involved in microtubule binding. A microtubule overlay assay has shown that TAN1 binds microtubules in vitro and antibodies raised against TAN1 bind to those cytoskeletal structures misoriented in *tan1* mutants. We have identified a gene in *Arabidopsis thaliana*, which encodes a protein that is highly related to TAN1. We call this gene *ATN* and have identified plants containing both T-DNA and DS element insertions in this gene. We have begun characterizing these insertion alleles. We are also performing experiments to determine the localization of ATN.

142 The Extensin Protein RSH is Required for Normal Cell Wall Growth and Development in *Aribidopsis*

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The complex wall surrounding every plant cell provides rigidity and form, and thereby plays an essential role in cell shape and in plant architecture. Although the molecular components of plant cell walls are considered identified, the exact function(s) contributed by each component is unknown. We study the hydroxyproline-rich glycoprotein (HRGP) RSH by comparing a rsh (root, shoot, hypocotyl defective) knock-out mutant to wild-type Arabidopsis. Previously we showed that RSH plays a crucial role in plant growth and development, by demonstrating abnormal cell shapes, and incorrect positioning of the cell plate during cytokinesis in the mutant. We demonstrated that RSH, an extensin type HRGP, whose expression is developmentally regulated, localizes to the cell wall in embryo and root cells. In the rapidly dividing embryo cells RSH localizes to both the mother cell walls and to the cell plate, and concentrates at the junctions of the new cell wall to their mother cell. In this poster, data is presented from transmission electron microscopy comparisons of the rsh mutant embryo and root tissue with that of wild-type. Our findings show that RSH is essential for cell wall integrity and normal wall growth. These results together with our earlier findings support the conclusion that the cell wall HRGP RSH is an essential component of plant cell wall structure and function.

143 Plant Cell Genomics Research in the High School Classroom

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Plant cell wall genomics research can be used to introduce topics in plant development, molecular and genetic analysis, electron microscopy and bioinformatics to high school students. In effort to introduce plant genomic research to the community, more than twenty high school students have become involved in our cell wall genomics project. Students learn about gene expression, plant transformation, and gene disruption. At the University, the SALK T-DNA seed lines are selected for homozygous gene disruption of cell wall related genes by a high-throughput system. These seed lines are selected for least insertion number and bulked up to be analyzed by collaborators and sent to the ABRC stock center. The high school students participate in seedling screens of these T-DNA lines using variable media including different carbon sources and different pHs. Students plate and grow coded seed lines, observe the developing seedlings and record findings. Currently experiments are done on a series of pHs; however, students can design independent projects to look at different aspects of plant growth. This arrangement gives the students an introduction to modern research approaches and the science of discovery. There are multiple replicates and consequently the data collected by the students can be considered reliable observations. As differences are observed, then the control seedlings and mutants are fixed for sectioning and scanning electron microscopy. Students are invited in small groups to the lab to observe the high throughput operation and further analysis of their "mutant". This work was supported by NSF # DBI-0077719

144 An Arabidopsis PIMT gene encodes two nuclear localized proteins.

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Age- and stress-related damage of protein can manifest itself as spontaneous deamidation and/or isomerization of asparaginyl or aspartyl residues, respectively. The activity of protein L-isoaspartyl (D-aspartyl) methyltransferase (EC 2.1.1.77) (PIMT) represents a cellular mechanism capable of correcting this damage. PIMT activity has been detected in almost all kingdoms. In *E .coli*, nematodes, and mice, PIMT activity is indispensable for life. In addition to a previously characterized PIMT in *Arabidopsis thaliana* (At3G48330; Mudgett and Clarke, 1996. Plant Mol Biol 30, 723-737) a second PIMT gene has been identified on chromosome 5. The gene on chromosome 5 (At5G50240; *PIMT2*) produces two transcripts *PIMT2* and *PIMT2′* through alternative splice site selection. We have characterized the expression of this gene and the localization of its gene products. Recombinant protein from both cDNAs has PIMT activity. Using multiplex PCR/AFLP, the transcripts were detected in leaves (both cauline- and rosette-leaves) and in stem tissues but there were no detectable transcripts in root tissues. The relative abundance of *PIMT* transcripts (*PIMT2=PIMT2′*) is invariant in leaf and stem tissues. Subcellular localization using cell fractionation followed by immunoblot analysis, as well as confocal visualization of PIMT-GFP fusions, demonstrated that the nuclear localization signal present in the amino terminus of PIMT2 and PIMT2' is functional.

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145 The role of organelles in cell death regulatory mechanisms in Arabidopsis

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In both plants and animals, programmed cell death (PCD) is a common defense response to pathogens and also occurs during development. Accumulating evidence suggests that many dying plant cells undergo the morphological and biochemical changes similar to those in apoptotic mammalian cells, but the death-initiating pathway that regulates PCD remain unclear. To elucidate the steps required for regulating and executing PCD in plants, we are using the characterized Arabisopsis *accelerated cell death* (*acd*) mutants to perform detailed cell biological analysis of cell death. We previously showed that one such mutant, called *acd2*, showed spontaneous spreading PCD that is light-dependent. ACD2 encodes red chlorophyll catabolite (RCC) reductase and suppresses the spread of disease. Early events of organelle alterations were investigated in this mutant and in wild-type cells treated with different cell death elicitors. We report here that organelles of the dying cells in *acd2* manifest biochemical changes including mitochondrial membrane permeabilization, although no dramatic ultrastructural changes are observed at an early stage after cell death induction. We will show that cell death in *acd2* and wild-type cells treated with elicitors has apoptotic features. We will also report our progress in monitoring the timing of organellar changes detected relative to cell death.

146 Microarray Analysis of Transgenic *Arabidopsis* Expressing Antisense *FtsZ*

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In order to understand the mechanisms involved in the regulation of chloroplast division and expansion, we are studying the transcriptional profiles of transgenic *Arabidopsis* lines expressing antisense *AtFtsZ1-1* and *AtFtsZ2-1* transgenes in a wild-type Columbia background. These plants are defective in chloroplast division, exhibiting drastically reduced numbers of greatly enlarged chloroplasts. Reporter gene expression patterns in transgenic plants and real-time RT-PCR assays indicate that *AtFtsZ1-1* and *AtFtsZ2-1* are coordinately expressed in young leaf tissue, roots, and in the shoot apex, and that their transcript levels fall dramatically in older leaf tissue. Based on these data, total RNA was isolated from the shoots of 10-day old plate-grown seedlings and used to generate cRNA for hybridization to Affymetrix ATH/1™ whole genome chips. Preliminary analysis indicates there are a number of genes in the antisense plants whose expression levels differ two-fold or more from those in wild type. Many of these genes are annotated as putative or hypothetical. Interestingly, there appears to be a small subset of genes that are consistently up- or down-regulated in both the *AtFtsZ1-1* and *AtFtsZ2-1* antisense lines. Future analysis of these genes may provide insight into the mechanisms governing the sensing and regulation of chloroplast volume in plants.

147 Analysis of AtAPG8 family proteins involved in Autophagy, a vacuolar degradation process

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Autophagy is an intracellular process for vacuolar degradation of cytoplasmic components. In yeast, we previously found that the C-terminus of Apg8 protein is modified with a lipid molecule by ubiquitination-like reaction after cleavage of its C-terminus by APG4 protease. These APG8 lipidation reactions are essential for yeast autophagy. Arabidopsis homologues of APG8 and APG4 (AtAPG8s, AtAPG4s) are supposed to function in a similar way in plant autophagy. All of nine *AtAPG8* and two *AtAPG4* were expressed ubiquitously in Arabidopsis and were further induced by nitrogen-starvation. Yeast Apg8 is known to be delivered to the vacuole as a consequence of autophagic process. This time, transgenic Arabidopsis expressing GFP-AtAPG8 fusion protein were observed by fluorescence microscopy. GFP-AtAPG8s, which are localized on some dot or ring structures in the cytoplasm, were delivered to the lumens of vacuole under nitrogen-starvation condition. This will provide a monitoring system assessing autophagic process in a whole plant. We will also discuss the role of AtAPG4 on this behavior of AtAPG8.

148 Multi-species analysis of centromere sequence in the Brassicaceae

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The goal of our research is clarifying the composition, organization and evolution of plant centromeres, the large DNA domains that mediate chromosome partitioning and inheritance. Although centromere function is conserved among all eukaryotes, centromere sequences are highly diverse. Large quantities of centromere sequence have been collected from only a few multicellular organisms (*Drosophila*, human, and the plant *Arabidopsis*). Interestingly, these efforts have revealed common sequence features (long arrays of repeats, insertion elements, and simple sequence repeats), yet different primary DNA sequences. Thus, clarifying the patterns of centromere evolution requires sequence comparison using close relatives.

To this end, we are conducting a comparison of centromere sequences from relatives of *Arabidopsis thaliana*, distinguishing rapidly evolving sequences from those that are conserved. So far we have chosen species that have diverged from *Arabidopsis* less than 20 million years, including *Capsella rubella*, *Olimarabidopsis pumila*, *Arabidopsis aerenosa*, and *Sisymbrium irio*. We will present our project strategies, including considerations for picking new species for centromere studies, generating BAC libraries, purifying centromere BACs based on their methylation patterns, and obtaining centromere shotgun sequence reads. We have initiated our study with *C. rubella*, and will present our preliminary sequencing results, which involve developing databases of centromere sequence to determine classes of centromere sequence, their abundance, and sequence conservation in and between species. Through this work we hope to generate an unprecedented compositional view of plant centromeres.

149 Analysis of an Arabidopsis ORFeome Collection Using Genome Tiling Arrays

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The most important product of the completion of the sequencing of the Arabidopsis genome is a complete and accurate catalog of genes and their products. Initially, the Arabidopsis genome was estimated to contain 25,500 protein-coding genes. In this study, our goals are (i) to construct a complete set of the Arabidopsis open reading frames (ORFeome by analogy to genome, transcriptome and proteome), (ii) to provide a complete collection of error-free ORF clones as a standard reagent to study the function of each and every gene in the Arabidopsis genome, and (iii) to develop empirical tools to validate the genomic sequence annotations. Towards our goals, we have designed two sets of high-density oligonucleotide tiling arrays which interrogate 5Mb regions of the Arabidopsis chromosomes 1 and 2. Using biotin-labeled cRNAs prepared from different tissue samples, we are able to construct a transcription map representing 4% of the genome. The transcription map provides unique information on not only transcriptionally active sites in the regions, but also transcription unit of each of predicted gene models (initial and terminal exons, exon/intron boundary, and 5' and 3' UTRs). In our assay, we found 77% of the annotated genes that have experimental evidence for expression such as ESTs and/or cDNAs were detected. In addition, 28% of the annotated genes which have no experimental evidence for their presence were also detected. The advantages of our approach include the experimental validation of the predicted gene models, the empirical information regarding 5' and 3' UTRs, the identification of novel transcripts not yet annotated by computational methods, and the detection of new species of non-coding regulatory RNAs. For more information, visit our web site at http://signal.salk.edu.

150 A sequence-based map of Arabidopsis genes with mutant phenotypes

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The classical genetic map of Arabidopsis contains 462 genes with mutant phenotypes. Chromosomal locations of these genes have been determined over the past 25 years based on recombination frequencies with visible and molecular markers. The most recent update of the classical map was published in a special genome issue of Science (1998; 282:663) that dealt with Arabidopsis. We present here a comprehensive list and sequence-based map of 620 cloned genes with mutant phenotypes. This map was recently published (Plant Physiol. 2003; 131: 409-418) and documents for the first time the exact locations of large numbers of Arabidopsis genes that give a mutant phenotype when disrupted by mutation. Such a community-based physical map should have broad applications in Arabidopsis research and should serve as a long-term replacement for the classical genetic map. Updated versions of this map will soon be available through TAIR. Please stop by this poster and check to see if your favorite mutant genes of interest are included on the map. Or check the supplemental information associated with the published paper at the Plant Physiology web site (www.plantphysiol.org) before the meeting. Forms will be available with the poster to submit additions for inclusion on the next scheduled update.

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151 Genetic analysis of condensin (SMC) mutants with a *titan* seed phenotype

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SMC proteins are chromosomal ATPases required for structural maintenance of chromosomes. Two different heterodimers have been identified: SMC2/SMC4 condensins and SMC1/SMC3 cohesins. Arabidopsis mutants disrupted in cohesin (*TTN7*, *TTN8*) or condensin (*TTN3*) genes exhibit a *titan* seed phenotype with giant endosperm nuclei (Liu et al. 2002. Plant J. 29: 405-416). Mutations in several unrelated genes also result in a *titan* phenotype (Tzafrir et al. 2002. Plant Physiol. 128: 38-51). Here we describe the isolation and characterization of additional SMC knockouts and present an updated model on condensin function in seed development.

Arabidopsis contains two putative SMC2 genes: *TTN3* (A) and *AtSMC2* (B). The original *ttn3* mutation results in a variety of endosperm defects but allows embryo development to proceed. This mutant allele (a) does not appear to be a null despite the presence of a T-DNA insertion within an intron. Using reverse genetics, we identified an *AtSMC2* knockout (bb) with a normal seed phenotype from the Salk collection. This result was inconsistent with our initial model that suggested a critical role for *AtSMC2* in embryo development. A double heterozygote (AaBb) was then constructed. As expected, seeds homozygous for both mutations (aabb) aborted early in development, but so did seeds homozygous for one mutation and heterozygous for the other (aaBb). Plants with the complementary genotype (Aabb) produced aborted seeds with variable phenotypes. Gametes carrying both mutant alleles (ab) also exhibited reduced transmission, depending in part on parental genotype. The condensin null phenotype in Arabidopsis may therefore be a combination of gametophytic lethality and seed abortion. This model is supported by efforts to isolate knockouts in the single *AtSMC4* gene. The only mutant identified to date exhibits a complex pattern of inheritance characterized by seed abortion and poor transmission. Recovery of additional knockouts may be limited by gametophytic lethality. These results illustrate some of the challenges faced when interpreting null phenotypes of essential genes.

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152 Exploring Patterns of Insertional Mutagenesis in Arabidopsis Thaliana

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Insertional mutagenesis techniques, including transposon- and T-DNA-mediated mutagenesis, are key resources for systematic identification of gene function in the model plant species *Arabidopsis thaliana*. We have developed an *Arabidopsis thaliana* insertion database (ATIDB) for archiving, searching and analyzing insertional mutagenesis lines (http://atidb.cshl.org). Users can search the database to identify insertions in a particular gene and the gene knockouts, or perform genome-wide analysis to study the patterns of insertional mutagenesis in *Arabidopsis thaliana*. Based on the output of ATIDB, we have performed a systematic analysis of the insertion sites of four major insertional mutagenesis agents in *Arabidopsis thaliana*: Ds and dSpm maize transposable elements as well as SIGnAL and GABI-Kat *Agrobacterium* T-DNAs. The transposons show marked preference for high GC content, in contrast to the T-DNAs which show no such preference. The transposable elements show a bias towards insertion near the translational start sites of genes, while the T-DNAs show a predilection for the putative transcriptional regulatory regions of genes. These results suggest that transposon-based mutagenesis techniques may prove the most effective for obtaining gene disruptions and for generating gene traps, while the T-DNAs may be more effective for activation tagging and enhancer trapping. Using pattern discovery techniques, we have identified a set of nucleotide sequence motifs that are commonly found at the sites of the transposon insertions. These motifs may play a role in the observed insertion site preferences of the transposable elements. However, we did not find specific motifs affecting the T-DNA insertion sites. These observations and discoveries provide the basis for models that predict insertion sites and potential gene disruptions.

153 Is expansin 8 central to the control of light-regulated stem growth?

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Studying the kinetics of light-regulated stem growth in photomorphogenic mutants of Arabidopsis through the use of computerized CCD imaging has helped us to better envision the roles played here by the phytochromes (PNAS **96**: 14142-14146) and certain downstream signaling elements, particularly SPA1 (Plant Physiol **126**: 1291-1298). A central feature of this work is the developing view that light-controlled seedling growth actually occurs as a dynamic balance of light-dependent growth inhibitive and promotive processes (Curr Opin Plant Biol **4**: 436-440). Building upon the hypothesis that SPA1 is a nuclear-localized transcription factor that detectably promotes growth after 2 h of red-light irradiation, we performed a tightly-defined genome-wide analysis of expression using an Arabidopsis microarray in an attempt to identify gene products that are central to growth regulation through SPA1 action. We probed arrays with labeled cRNA synthesized from mRNA isolated from duplicate dark-grown and 3-h red-light-treated populations of both wild-type and SPA1-null seedlings. This analysis identified a small sample of genes that are regulated differently in red light between wild type and *spa1* mutants. One such gene with a clear role in cell expansion (i.e. growth) is Expansin 8 (*EXP8*). We are currently using RT-PCR as a means to verify the results of our genome survey. We are also using this technique to test the hypothesis that EXP8 is a central element in light-regulated growth by establishing whether its levels are differentially regulated by other phytochrome downstream signaling factors such as FHY1 and FHY3. These data and our ongoing progress will be discussed.

154 Control of NOR methylation in various accessions of *Arabidopsis thaliana*

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5-methylcytosine is the most common form of covalent DNA modification in eukaryotic organisms. Generally, high levels of 5-methylcytosine are associated with transcriptionally silent regions of the genome, while transcriptionally active regions usually lack DNA methylation. In addition, genomic regions exhibiting a high density of methylated cytosines are often heterochromatic and consist mainly of repetitive DNA sequences. In addition to the centromeres, these highly methylated, heterochromatic regions include the nucleolus organizer regions (NORs), which contain long arrays of ribosomal RNA (rRNA) genes. In the flowering plant *Arabidopsis thaliana*, the NORs show high levels of variation in DNA methylation patterns, with 5-methylcytosine levels ranging from 20% to 90% of all cytosines. We previously reported the results of a study examining the mechanisms controlling cytosine methylation levels at the NOR in the Cape Verde Island and Landsberg strains, where we identified *cis*- as well as *trans*-acting control elements in addition to epigenetic influences. Here, we present the results of a follow-up study investigating these mechanisms in two additional crosses between high and low NOR methylation accessions, Columbia x Cape Verde Island and Canary Island x Columbia. NOR methylation levels appear to be controlled by similar mechanisms in all crosses examined; however, the level of control exerted by each mechanism appears to differ between the crosses. We present a model incorporating the various components involved in the control of NOR methylation and propose experiments to test it.

155 Telomeres and telomere-associated factors in Arabidopsis

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Telomeres are physical ends of linear eukaryotic chromosomes required to form a protective cap. In fission yeast, the Pot1 protein physically binds to the 3? single strand overhang of telomeres. *S.pombe* mutants that survive the loss of Pot1 protein circularize their chromosomes. Sequence homologues of *S.pombe* Pot1 protein have been found in other organisms, but little is known about their function in higher eukaryotes. We have recently identified two homologues of Pot1 in *Arabidopsis* (AtPot1 and AtPot2). These genes have 33% sequence identity (56% similarity) within the predicted DNA binding domains. The genes are ubiquitously expressed at low levels. *E.coli*-expressed AtPot1 and AtPot2 proteins bind single-stranded telomeric DNA specifically, with five telomere repeats being the best substrate for binding. Yeast two-hybrid and co-immunoprecipitation experiments indicate AtPot1 and AtPot2 can form homo- and heterodimers. Analysis of RNAi and T-DNA mutants is underway to determine the effects of AtPot1 and AtPot2 deficiency on telomere biology and lifespan of *Arabidopsis*. We also analyzed telomere length distribution in *Arabidopsis* ecotypes used to generate knock-outs, activation lines and T-DNA tagged mutants. Telomeres in plants of the Columbia ecotype and five other ecotypes display telomeres in the range of 2-5 kb. In contrast, plants of Cvi-0 and Nd-0 origin carry telomeres twice as long. Surprisingly, WS plants can be separated into two distinct groups: those with telomeres of 2-5 kb and those with telomeres of 4-7 kb. To learn more about telomere dynamics, we analyzed the behavior of subtelomeres in individual plants. Our data indicate that telomerase?s action on individual chromosome ends is stochastic, and each individual telomere substrate is extended differently by telomerase. Despite the stochastic nature of telomere elongation, the overall length of the telomere tract is strictly regulated within an ecotype-specific range. Our data support the conclusion that the length of each telomere is established early in development and is maintained for the entire lifespan. The striking variability in telomere length among different individuals and different ecotypes has important implications for interpreting results of mutations in telomere maintenance machinery.

156 A New Reverse-Genetic Resource for Knocking-Out Small Genes and Tandemly Duplicated Genes

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It has become apparent that reverse-genetic analysis is a powerful method for analyzing gene function in Arabidopsis. Several resources are currently available that make knockout mutants of Arabidopsis genes easily available to researchers throughout the world. Because these systems are all based on random mutagenesis, the principles of probability dictate that null alleles for many small genes will not be available in the current collections. In addition, if you are interested in a family of tandemly duplicated genes, there is currently no simple system for creating a plant that carries knockout alleles for more than one of these loci. Because of functional redundancy, it is valuable to be able to create double- or triple-mutants that combine null alleles of closely related genes in a single plant.

We are currently in the process of developing a new reverse-genetic resource that should provide the means to obtain knockout alleles for genes that have escaped mutagenesis in the currently available reverse-genetic collections. In addition, the system also provides the means to knockout multiple members of a tandemly duplicated set of genes. The resource is based on a new collection of T-DNA lines that were constructed using a new T-DNA vector that contains a DS transposon. We are using TAIL PCR to map the locations of thousands of individual T-DNA inserts from this collection. The DS transposon in this vector can be mobilized by crossing the T-DNA line to a source of transposase. Because DS transposition is biased towards tightly linked regions of the genome, it should be possible to saturate a local area of the genome with transposed DS elements, thereby generating a knockout of the small gene of interest. This vector also features two LoxP sites, one in the T-DNA and one in the DS element. These LoxP sites can be used to create small chromosomal deletions that remove multiple members of a tandem gene array. The deletion reaction is triggered by crossing the T-DNA line to a plant that expresses the CRE recombinase protein. Our progress in establishing this new resource for reverse-genetics will be reported in our poster.

157 The Arabidopsis Cohesin Proteins Play Essential Roles in Mitosis and Meiosis

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The faithful transmission of chromosomes during mitosis and meiosis requires the establishment and subsequent release of cohesion between replicated chromosomes. Sister chromatid cohesion is mediated in large part by the cohesin complex, which consists of four highly conserved proteins: SMC1, SMC3, SCC1/REC8 and SCC3. Mitotic cohesin complexes contain SSC1, while meiotic cohesin complexes contain the related REC8 protein. While the cohesin complex has been studied extensively in several other organisms, very little is know about these important proteins in plants. Our laboratory is currently investigating a number of predicted Arabidopsis cohesin proteins to better understand their roles in plants. Inactivation of SYN1 results in defects in meiotic chromosome cohesion and condensation, which result in chromosome fragmentation and the formation of polyads. FISH experiments on microsporocytes from syn1 plants demonstrate that the mutation eliminates arm cohesion as early as interphase while centromere cohesion is maintained until approximately anaphase I. *SYN1* encodes a protein that localizes to arms of meiotic chromosomes from approximately meiotic interphase to anaphase I. It is not detected at the centromeres or after metaphase I. These results suggest that the major role of SYN1 is in meiotic arm cohesion. Three additional *SCC1/REC8* orthologs (*SYN2*, *SYN3* and *SYN4*) have been isolated and are being characterized. SYN3 appears to have a novel role in the plant nucleolus, while SYN2 and SYN4 may participate in mitosis. Results from SYN2, SYN3 and SYN4 immunolocalization experiments and the analysis of *syn2*, *syn3* and *syn4* mutant plants will be presented. In order to better understand plant cohesin complexes and further investigate how plants facilitate centromeric cohesion, we have isolated and characterized AtSMC1 and AtSMC3. Antibody to AtSMC3 strongly labels meiotic chromosomes from interphase to diakinesis at which time most of the protein relocalizes to the spindles until late telophase II. Finally AtSMC3 RNAi plants have been generated and are being analyzed in order to circumvent difficulties associated with the embryo-lethality observed in SMC1 and SMC3 plants.

158 The Arabidopsis MALE MEIOCYTE DEATH1 gene encodes a PHD-finger protein that is required for male meiosis

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Reproductive development requires normal meiosis, which involves several highly coordinated events that are regulated a number of ways, including transcriptional, and cell cycle control mechanisms. Although a number of mutations affecting different aspects of meiosis have been characterized in plants, very little is known about the regulation of plant meiosis at the molecular level. In particular, no meiosis-specific transcriptional regulators have been identified in plants and checkpoint control has not been observed during plant meiosis. We report here the isolation and characterization of a new Arabidopsis male sterile mutant that exhibited meiotic defects. Meiocytes from mutant plants appeared normal up to diakinesis when they exhibited signs of apoptosis, including defects in chromosome behavior, cytoplasmic shrinkage and chromatin fragmentation followed by cell death prior to cytokinesis. Therefore, the mutant was named *male meiocyte death1* (*mmd1*). The *MMD1* gene was cloned using Ds transposon tagging and encodes a PHD domain containing protein. *MMD1* is expressed preferentially during male meiosis. Our results suggest that MMD1 may be involved in the regulation of gene expression during meiosis, and that the mmd1 mutation triggers cell death in male meiocytes.

159 Transcript Profiling of Arabidopsis thaliana P450s

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In plants, cytochrome P450 monooxygenases function in the biosynthesis of lignins, pigments, defense compounds, fatty acids, hormones and growth regulators as well as in the metabolism of herbicides, insecticides and pollutants. Towards defining the function of the 271 P450 genes existing in *Arabidopsis*, we have constructed microarrays containing gene-specific elements for 265 P450s, 40 biochemical pathway markers and a number of stress-responsive transcription factors and EST elements for 322 physiological function markers and analyzed these with probes representing transcripts derived from individual tissues of 7-day-old and 1-month-old plants. Accompanied by RT-PCR analysis of the transcript levels derived from individual loci, these microarrays have highlighted a number of P450 transcripts expressed in most, if not all tissues, under normal growth conditions and detailed the wise array of P450 transcripts expressed at some constitutive level in one or more of these tissues. Comparative profiling of 7-day-old seedling transcript levels on the 8000-element *Arabidopsis* oligoarray has clarified the extent to which these microarrays and the smaller of the currently available oligoarrays can be compared for expression profiling of P450 genes. These P450 microarrays are currently being used to define the P450 transcript profiles in seedlings exposed to a variety of chemicals including plant signaling molecules (JA, SA), phenobarbital, herbicides (DICAMBA, atrazine) and fungal defense activators (BTH) and environmental stresses including osmotic and cold stresses. Together, these analyses detail the unique and overlapping responses of P450 loci and biochemical pathway loci to internal and external chemical cues. A number of biochemical and genetic strategies are being used to functionally define the substrate specificities of the P450s induced or repressed by these stresses.

160 The Carotenoid Cleavage Dioxygenases of *Arabidopsis thaliana*

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Apocarotenoids are a class of secondary plant metabolites derived from the cleavage of carotenoids. They are found in plants, animals, and photosynthetic bacteria. The function of apocarotenoids are varied. Volatile apocarotenoids, like β-ionone, contribute to the flavor and aroma of fruits and vegetables. Other apocarotenoids are biologically active. For example, retinal is required for vision in animals and the apocarotenoid-derived plant hormone, abscisic acid, is required for seed dormancy and drought stress tolerance. A family of nine proteins, whose members are homologous to a known carotenoid cleavage enzyme from maize, VP14, has been identified in the Arabidopsis genome. Five of the Arabidopsis proteins, like VP14, cleave 9-cis epoxycarotenoids and are therefore called 9-cis epoxycarotenoid dioxygenases (NCED). The remaining four are unlikely to be NCEDs and are instead termed Carotenoid Cleavage Dioxygenases (CCD). The research to be presented focuses on the characterization of three of the Arabidopsis CCDs. Carotenoids are mainly concentrated within the plastid. Therefore, localization of each CCD to and within the chloroplast has been determined. Both CCD7 and CCD8 are targeted to the chloroplast stroma, whereas, CCD1 is not chloroplast targeted. Transcript expression patterns have also been determined. CCD7 and CCD8 are expressed mostly in the inflorescence and root tissue, respectively. Again, CCD1 differs from CCD7 and CCD8 in that it is expressed fairly uniformly throughout the plant. In order to define the functions of the CCDs in vivo, loss-of-function mutants have been isolated. Single knockouts for CCD7 and CCD8 each display a branching phenotype. The *ccd1* knockout does not have an obvious phenotype under normal growing conditions. The phenotypes of the *ccd7* and *ccd8* loss-of-function mutants tell us that these gene products play an important role in plant development. Recent work shows that *ccd8* is allelic to *max4*, a known branching mutant. The apocarotenoids produced by the action of these CCDs may be biologically active compounds involved in the maintenance of apical dominance. Further investigation into their modes of action may also answer basic questions regarding plant growth and development.

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161 Cloning, Spatial Expression, and the Enzymatic Properties of Arabidopsis Secretory Phospholipase A_2

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Secretory phospholipases A_2 ($sPLA_2$) hydrolyze membrane phospholipids at the *sn*-2 position to release free fatty acids and lysophospholipids. As is known in animals, plant $sPLA_2$ probably also play important roles in growth and development, but their cellular functions are poorly understood because of a lack of cloned and identified $sPLA_2$ genes. Using the previously published carnation $sPLA_2$ sequence as a query, we identified four homologs of the predicted cDNA encoding $sPLA_2$ in the Arabidopsis genomic database at the National Center for Biotechnology Institute (NCBI). We cloned one homolog corresponding to At4g29460, which we named Arabidopsis secretory phospholipase A_2-gamma ($AtsPLA_2$-γ), and examined its enzymatic properties. The full-length cDNA sequences of $AtsPLA_2$-γ was found to contain 791 nucleotides and an open reading frame consisting of 564 nucleotides, which encodes a protein of 187 amino acids containing the predicted signal peptides of 25 amino acids. The resultant mature protein was found to contain 162 amino acids and to have a calculated molecular mass and PI of 17.5 kDa and 8.25, respectively. A comparison of its deduced amino acid sequences revealed that $AtsPLA_2$-γ has low homology with other plant $sPLA_2$s in terms of its overall amino acid sequence, but high homology at the catalytic site and the Ca^{2+}-binding loop. The recombinant protein of $AtsPLA_2$-γ showed maximal enzyme activity at pH 8.0, and like other $sPLA_2$s required Ca^{2+} for activity. $AtsPLA_2$-γ showed *sn*-2 position specificity but no prominent acyl preference, though it showed head group specificity to phosphatidylethanolamine (PE) rather than to phosphatidylcholine (PC). By quantitative RT-PCR, the expression of $AtsPLA_2$-γ predominated in the mature flower rather than in other tissues, such as, cauline leaves, rosette leaves, inflorescences, and roots. Subcellular localization analysis confirmed the secretion of $AtsPLA_2$-γ into the intercellular space. Thus, the biological and biochemical data obtained confirmed this protein as a $sPLA_2$, which may regulate phospholipid-derived signal transduction.

GenBankTM/EBI Data Bank accession number(s) is AY148346.

162 RNA Expression Profiling on Bead-based Microarrays

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We have developed a new gene expression profiling system based on bead-based fiberoptic microarrays. This system allows gene expression profiles to be obtained for ~ 700 genes from 96 samples at a time, enabling thousands of samples to be processed a day. The system consists of 96 individual microarrays arranged in an 8-by-12 matrix that matches the well spacing of a standard microtiter plate. Each individual array in the matrix holds ~ 1,500 different oligonucleotide probes. The probes are attached to 3 micron beads, which are assembled into wells at the end of an optical fiber bundle to make an array. Since there are many more wells than probe sequences, multiple copies of each bead are present in the array. This built-in redundancy improves robustness and measurement precision. A series of experiments using spiked transcripts have demonstrated that our platform performs at a high level which compares favorably to other platforms. By measuring cell-specific gene expression in samples of mouse B and T cell RNA mixed at various ratios, we show that this high level of performance extends to endogenous eukaryotic genes. In addition, this high level of performance has been achieved using drastically reduced levels of starting material using standard sample preparation protocols. The combination of hundreds of genes per array and the sample throughput of the matrix format should enable “many genes, many samples” applications that are not adequately addressed by current gene expression technologies.

163 Characterisation and expression of lipoxygenases and hydroperoxide lyases.

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We are over-expressing lipoxygenases (LOXs) B from *Medicago truncatula* and 2 and 3 from *Pisum sativum*, and hydroperoxide lyases (HPLs) E and F from *M. truncatula*, in *Arabidopsis thaliana*, to modify oxylipin metabolism. All sequences were expressed in *E. coli* as Gateway™ clones, characterised with respect to kinetic properties and substrate/product specificity, and transferred directly into pGreen transformation vectors, using the Gateway™ technology, for Arabidopsis over-expression. The HPLs were constructed as His-tag fusions with (+) and without (-) an N-terminal sequence that has been proposed to have a regulatory function.

HPLF+ is a member of the CYP74C class of cytochrome P450s, has a pH optimum of 6.5-7.0, is most active with the 13-hydroperoxides of linoleic and α-linolenic acids but is also active on 9-hydroperoxides, and has a very high turnover number (~750 sec^{-1}). Activity was the same whether or not the N-terminal 11 amino-acid "regulatory" sequence was present. HPLF is most similar to melon and cucumber HPLs, and has been shown by GC-MS to possess hydroperoxide lyase activity. Highly monodispersed preparations of HPLF+ are being subjected to crystallisation trials for structural determination.

HPLE has provisionally been designated a 13-specific HPL; its sequence places it in the CYP74B group of P450s and it is 97% identical to a previously characterised HPL from *Medicago sativa*. Both HPLs are being introduced into Arabidopsis under the control of a leaf-specific promoter.

LOX B and LOX-3 both show dual specificity with linoleic acid, forming 9- and 13-hydroperoxides, and generate keto-fatty acids that are believed to arise from a reaction intermediate that is normally converted into hydroperoxide. LOX-2 is a 13-LOX that does not generate keto-acids. All three have been expressed in *E. coli*, enzymologically characterised and transformed into Arabidopsis under the control of CaMV 35S promoter.

None of the LOX or HPL sequences has an N-terminal targeting sequence and they are expected to be located in the cytosol in the transgenic Arabidopsis. We will assess the consequences of their over-expression for oxylipin metabolism and will combine the over-expression of LOX, HPL and other enzymes of oxylipin metabolism.

164 Similar domains, different substrates: a reverse genetics approach to characterize a glycosyltransferase in A. thaliana

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Glycosyltransferases (GTs) transfer sugar moieties from activated sugars to a variety of acceptors, e.g. proteins, lipids and other sugars. 413 putative GTs are annotated in *A. thaliana*.Only a few are functionally characterized. Characterization of GTs is challenging: Firstly, the many possible putative substrates hamper the development of suitable biochemical assays. Secondly, sequence similarities often do not allow conclusions about possible functions.(Keegstra and Raikhel 2001) Most of the GTs described at the molecular level are either cellulose synthases or participate in primary metabolism. Recently, three GTs involved in hemicellulose synthesis were characterized at the molecular level. (Faik et al. 2000; Edwards et al. 2002; Faik et al. 2002) We chose a reverse genetics approach to characterize a putative GT in *A. thaliana*. A member of glycosyltransferase family 8 was chosen after an *in silico* analysis in which it turned out to be the only putative plastidic member in its family. T-DNA insertion lines have been isolated, however, they do not display an apparent phenotype. After confirming plastidic localisation by 35S::full-length::GFP fusion, antibodies were raised for further experiments. Using promotor::GUS fusions , we observed expression in the heart of the rosette and along vascular tissues into the petioles and the root. The reporter studies have been confirmed by RNA gel blot hybridisation and RT-PCR. The information obtained so far allows us to narrow the possible substrates of the putative GT down.

Edwards, M. E.(2002) et al. Plant Physiol 129(3): 1391-7.
Faik, A. et al. (2000). J Biol Chem 275(20): 15082-9.
Faik, A. et al. (2002) Proc Natl Acad Sci U S A 99(11): 7797-802.
Keegstra, K. and N. Raikhel (2001) Curr Opin Plant Biol 4(3): 219-24.

165 Highly divergent tocopherol methyltransferases in photosynthetic organisms

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Tocopherols are important human nutrients and are only synthesized in photosynthetic organisms. The tocopherol pathway and most pathway enzymes are highly conserved in cyanobacteria and plants. The only exception is 2-methyl-6-phytyl-1,4- benzoquinone/ 2-methyl-6-solanyl-1,4-benzoquinone methyltransferase (MPBQ/MSBQ MT), which catalyzes key methylation steps in both tocopherol and plastoquinone (PQ) synthesis in plants. A combined genomic, genetic, and biochemical approach is employed to isolate and characterize VTE3 (vitamin E), which encodes the *Arabidopsis* MPBQ/MSBQ MT. The weaker, EMS-derived *vte3-1* allele exhibits altered tocopherol composition but has little impact on PQ levels. *vte3-2* T-DNA insertion allele is deficient in PQ and its tocopherol profile changed dramatically. Though highly divergent in primary sequence, VTE3 is a functional equivalent of *Synechocystis* MPBQ/MSBQ MT. *In vivo* studies of mutations in *Arabidopsis*- and *Synechocystis*-type MPBQ/MSBQ MT revealed important differences in enzymatic redundancy and in the regulation of tocopherol synthesis in the two organisms.

166 DPE2 - An extraplastidial enzyme of starch degradation

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Arabidopsis leaf starch undergoes a diurnal cycle of synthesis and degradation. This model system allows the elucidation of enzymatic components of the degradative pathway by studying mutants obtained by both forward and reverse genetic screens. Using reverse genetics, we have isolated a mutant (*dpe2-1*) that lacks a putative disproportionating enzyme. Preliminary results show that this is a novel cytosolic enzyme necessary for starch degradation. The profiles for starch, sugars and malto-oligosaccharides during the diurnal cycle of this knockout mutant will be described and the implications of these results in further clarifying the pathway of starch degradation in higher plants will be discussed.

167 Nanodisc assembly of P450 monooxygenases into soluble Nanodisc system for substrate binding analysis.

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Cytochrome P450 monooxygenases (P450s) represent a large enzyme superfamily involved in a multitude of biochemical pathways, many of which are activated in response to chemical, developmental, and environmental factors. The P450 enzymes are ubiquitous in nature and participate in physiologically important processes ranging from detoxification of xenobiotics to synthesis of biologically active compounds such as hormones, steroids, defense compounds, signaling molecules, and fatty acids. The *Arabidopsis* genome contains at least 271 P450 genes, most of which code for proteins translocated into the endoplasmic reticulum membrane and anchored by their N-terminal signal sequence. Membrane-bound proteins in their native form have consistently proven difficult to purify and characterize due to their poor solubility in aqueous environments. Recently, we have developed a technique that incorporates heterologously-expressed membrane proteins directly from cell membranes into soluble, monodisperse lipid nanobilayers (Nanodiscs™). Using this technology we have shown that P450s embedded in Nanodiscs™ retain a correctly configured active site and whose substrate binding capacities can be measured with classic type I binding analysis in a high throughput microtiter format. We are in the process of utilizing this technology to solubilize and array a number of *Arabidopsis* P450s in 96-well microtiter plates for screening of potential substrates and definition of the reactivities of these enzymes. The six P450 proteins that we have selected for use in this study are induced upon treatment of *Arabidopsis* plants with methyl jasmonate as defined by microarray analyses.

168 Structural Analysis of Flavonoid Enzymes in Arabidopsis

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Biosynthetic pathways are often organized within cells as groups of interacting enzymes or enzyme complexes. Although this organization appears to be widespread in biological systems, it is currently poorly understood. The flavonoid biosynthetic pathway of Arabidopsis thaliana has been developed in our laboratory as a model for studying enzyme complexes[1]. Several of the enzymes of this pathway have been shown to interact in vitro, and have also been found to be co-localized in roots[1,2]. In an effort to further the understanding of how these enzymes interact, it would be advantageous to have a three-dimensional picture of these enzymes interact with each other. Our studies are currently focusing on the first two steps in the flavonoid biosynthetic pathway, chalcone synthase (CHS) and chalcone isomerase (CHI), which catalyze the condensation reaction of coumryl-CoA and 3 malonyl-CoA into the main flavonoid backbone. We present in this poster a 1.7Å resolution structure of CHS and evidence for this complex using small angle neutron scattering (SANS). Initial data from SANS indicates that there are 2 CHI units interacting with 1 CHS homodimer in solution. This data will also be used to generate a three-dimensional solution structure of these enzymes in complex. It is our hope that SANS, coupled with X-ray crystallography data will provide information on the interfaces involved with the protein-protein interactions which can then be used as a rational approach to site-directed mutagenesis experiments to increase or decrease binding of these enzymes with one another.

1. Burbulis, I.E. & Winkel-Shirley, B. (1999) Proc. Natl. Acad. Sci. USA. 96:12929-12934.
2. Saslowsky, D.E. and Winkel-Shirley, B. Plant J. 27(1):37-48.

169 Arabidopsis thaliana transcription factor function search

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Plant transcription factors are central components of the regulation of many processes in plant biology, including cell development, differentiation, cell cycle, secondary metabolism, disease resistance and defence responses. Specific transcription factor families such as MYB domain proteins, bZIP proteins, WRKY proteins, bHLH proteins and some others are known to exist in plants. For the majority of these genes, however, little functional information is available.

Current research in many labs is aiming at the functional dissection of the role of a wide variety of plant transcription factors by means of molecular and genetic approaches. Our approach to assign functions to single members of large TF families is to systematically analyse overexpression- (OX), knock out- (KO) and promoter::GUS lines. We concentrate on three selected TF families which are known to control phenylpropanoid metabolism in plants, namely R2R3-MYB (helix-turn-helix), bZIP (basic region/leucine zipper), and bHLH (basic region/helix-loop-helix) domain proteins. Functions will be deduced from phenotypes of OX and KO lines, from the identification of target genes, and from data on interacting proteins. To provide a solid basis for our approach we analysed the near-complete genome of *A. thaliana* resulting in a significantly improved annotation of these three gene families. Full-length coding sequences for members of the three gene-families were produced by PCR techniques and confirmed by sequencing. Already at this stage of the project basic information on the expression pattern of any given gene was obtained by the parallel use of RT-PCR templates from different sources. The TF cDNAs were cloned as ENTRY clones suitable for the GATEWAY system. OX constructs for 100 TF genes have been generated and transformed into *A. thaliana* Columbia-0 plants and KO lines for 27 TF genes have been identified so far. We will present first results of our reverse genetics approach.

170 Heterologous Expression of Arabidopsis CYP73A5 in Insect Cells and Incorpor

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An *Arabidopsis* cytochrome P450 (cinnamate-4-hydroxylase, C4H, CYP73A5) has been expressed in a baculovirus expression system yielding correctly configured P450 detected by CO difference spectrum from either microsomes or cell lystes of baculovirus infected insect cells. *Arabidopsis* NADPH-cytochrome P450 reductase (ATR1) has also been cloned and expressed in this system. Microsomes and cell lysates of insect cells co-expressing CYP73A5 and ATR1 effectively hydroxylate cinnamate, the best substrate for this enzyme. To further studies in P450 substrate profiling, microsomal membrane proteins from insect cells overexpressing CYP73A5 has been incorporated into soluble Nanodiscs through a simple self-assembly process. The soluble nanoscale particles created in this process contain lipids stabilized by encircling Membrane Scaffold Proteins (MSPs) and membrane-bound proteins derived from the Sf9 microsomal membranes. Characterization of size-fractionated CYP73A5 containing Nanodiscs has shown that, after Nanodisc solubilization, the P450 target protein maintains its integrity and its ability to bind its substrate cinnamate. In summary, our data suggest the baculovirus expression and Nanodiscs assembly systems can be used for expressing plant P450s in soluble, native environments that maintain the spectrum of their substrate binding activities and present the opportunity for development of high-throughput substrate screening methodologies.

171 Molecular and Physiological Analysis of Arabidopsis Lax3 Gene

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The Auxin1-like permease 3 (Lax3) gene was RT-PCR cloned from the genome, sub-cloned in to yeast shuttle vector NEV-E and analyzed for functional properties. Preliminary yeast plate growth experiments suggest that Lax3 is a general amino acid transporter that has affinity to many amino acids with somewhat variation. We are currently working on C-14 labeled amino acid uptake assays to find out detailed substrate specificity. A transposon insertion mutant of Lax3 in Arabidopsis thaliana was obtained from Cold Spring Harbor Laboratories. The Southern analysis showed single transposon insertion in the genome, and TAIL-PCR and sequencing results confirmed the point of insertion being in the Lax3 gene. GUS staining results suggested Lax3 expression is mostly limited to vascular tissue at all stages of the plant life, although the place of expression varies temporally. Confirmation of spatial and temporal gene expression patterns are being done with RNA Gel-Blot analysis, RT-PCR, and Real-Time PCR. The mutant plant had no phenotype in various stress conditions tested. Additional experiments are being conducted to find out specific conditions this gene might be regulated in *Arabidopsis*.

172 Insertion Site Dependent GUS Expression Patterns in Putative GABA Permease

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We've identified twelve independent transposant lines with gene or enhancer traps inserted into the same, putative GABA permease in *Arabidopsis*. Transposon insertions were confirmed in seven of these lines. Of five enhancer trap lines, only three stained positively for GUS activity, and of five gene trap insertions tested, only one line displayed GUS staining. GUS expression patterns of the enhancer trap lines were totally different in all three lines, while expression of the gene trap insertion line was restricted to the vascular tissue starting from the 2 weeks old growth. The different patterns of GUS expression suggests the gene is controlled by different enhancer elements possibly associated with other genes as well. Of five gene trap lines, three are in the same orientation as the putative permease gene yet only one yields a staining pattern. Subsequent generations of this gene trap line, however, also displayed different GUS expression patterns. TAIL-PCR and Southern analysis of the individual gene trap plants displaying different GUS expression patterns showed different insertion points (possibly due to a cryptic AC transposase activity) in the same gene. We are currently testing these mutant plants for phenotype expression in Arabidopsis seedlings to confirm the differential gene expression *in situ*. We are also examining the transport activity of the putative GABA permease and its expression pattern with RNA-gel blots, RT-PCR, and Real Time-PCR.

173 The Arabidopsis Putative GABA Permease Doesn't Transport GABA in Yeast

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The *Arabidopsis thaliana* At2g01170 gene was putatively named as "gama amino butyric acid (GABA) permease" based on its significant similarity to yeast GABA permease (UGA4) gene. We have obtained the At2g01170 cDNA from Ohio Stock Center, cloned into yeast shuttle vector NEVE, and tested for amino acid uptake assays as well as plate growth experiments. Both results showed that At2g01170 does have amino acid transport activity for various amino acids but not for GABA. Unlike most amino acid transporters described so far, At2g01170 displayed both export and import activity for various amino acids. We are currently characterizing the gene function with respect to substrate specificity, Km and Vmax. The spatial and temporal expression patterns of the gene are being studied through GUS staining, RNA Gel-blot analysis, RT-PCR and Real-Time PCR.

174 *Arabidopsis thaliana clb6* albino mutant is affected in *LYTB* gene encoding the last enzyme of the isoprenoids MEP biosynthesis pathway

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The biosynthesis of isoprenoids has been a central topic for more than half a century. Plants synthesize a large number of isoprenoid compounds that are very diverse in structure and function including photosynthetic pigments, electron carriers, and growth regulators. Plant isoprenoids are formed by two independent pathways that operate in different subcellular compartments; the mevalonate pathway works in cytosol, whereas the recently discovered DXP or MEP pathway operates in plastids. We have isolated a collection of mutants affected in early chloroplast development that resulted in an albino seedling phenotype named as *chloroplast biogenesis (clb)*. Interestingly, in the vicinity where one of the *clb* mutants mapped *(clb6)*, the homologous *Arabidopsis LYTB* gene (AT4g34350), involved in the MEP isoprenoids biosynthesis pathway, is located. As other genes of the MEP pathway has been reported to render an albino phenotype, we considered the *LYTB* gene as a good candidate to be the gene affected in the *clb6* plant mutant. Our results showed that the introduction of the full *LYTB* cDNA in the *clb6* plant, is sufficient to rescue the albino phenotype. It was also corroborated that *clb6* mutant carries a point mutation that introduces a stop codon in the *LYTB* gene at position +1068 (aa 354), apparently resulting in the degradation of the *LYTB* transcript as *LYTB* mRNA is undetectable in the mutant plant. A detailed characterization of the *clb6* mutant compared to wild type plant, including the expression of the other genes from the MEP pathway at the messager and protein level will be presented. Our results demonstrate that the AT4g34350 open reading frame represent the functional *LYTB* gene responsible of the last step of the isoprenoids biosynthesis in *Arabidopsis*.

175 Positional cloning of the *CER7* gene of *Arabidopsis*

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Plant cuticular waxes embed and overlay the cutin matrix of higher plant cuticles. By forming the primary hydrophobic barrier and interface between the plant and the enviroment, they play important roles in plant responses to biotic and abiotic factors. A number of waxless (Cer) mutants have been isolated from *Arabidopsis*. The *cer* mutations identify gene functions required for wax deposition and allow cloning of these genes. Thus, they are an invaluable resource for increasing our understanding of wax production. We have isolated the *CER7* gene using a positional cloning approach. We fine-mapped the chromosomal location of the gene using simple sequence length polymorphism (SSLP) marker analysis of an F_2 mapping population, and complemented the mutation using a genomic fragment including the *CER7* coding sequence, 1.4 kb of 5' sequence and 300bp of 3' sequence. We have also characterized a SALK institute T-DNA insertion line with an insertion site 400bp 5' to the *CER7* translational start codon, which also has a waxless phenotype. Phenotypic and genetic analyses of the two mutants, as well as an analysis of the *CER7* expression pattern, will be presented. Potential roles for the CER7 protein in wax synthesis will be discussed.

176 Metabolic Networks in Monolignol (Radical Radical) Coupling in Arabidopsis

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The arabidopsis genome consists of at least sixteen isovariants of the monolignol radical-radical coupling proteins, defined as dirigent and dirigent-like proteins, respectively (Latin: *dirigere*, to guide and/or align). One objective of the ongoing study is to define the physiological roles of each of the dirigent protein isovariants, including those of any related homologues awaiting discovery. Together, the 16 isovariants appear to constitute a comprehensive monolignol radical radical coupling network operative in different cells and tissue types during the life cycle of arabidopsis. Some of the genes are anticipated to encode proteins involved in lignan formation for plant defense, whereas others are presumed to harbor arrays of multiple dirigent (monolignol binding) sites considered to be involved in lignin biosynthesis. The 16 isovariants have 29-65% sequence similarity to the *Forsythia dirigent* gene (GenBank AF210061). The experimental strategy employed thus far was designed to establish the spatial and temporal expression pattern of each of the 16 isovariants. This was achieved by constructing two sets of different expression plasmids consisting of 1) 16 equivalent *dirigent* promoter::*gus plus*::3'flanking sequence::T-nos and 2) 16 equivalent *dirigent* promoter::*mgfp*::3'flanking sequence::T-nos. Histochemical analyses of gus and gfp expression shows that the majority of *dirigent* isovariants are expressed in the vascular system during development but with differing levels of expression. For example, *AtDir10*-GUS was expressed throughout all of the developmental stages; GUS activity was observed in the main vein and trichomes of leaves, anthers and stigma of mature flowers, as well as in the vascular bundles of stems and roots. Molecular, biochemical and histochemical analyses of *gus* and *gfp* expression reveal further the existence of a complex network controlling monolignol radical-radical coupling. Supported by NSF (MCB-0117260) and NASA (NAG2-1513).

177 Differential expression of glutamine synthetase and glutamate synthase gene in Arabidopsis root

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In higher plants, NH_4^+ can be released from various metabolic processes, such as nitrate assimilation, photorespiration, amino acid catabolism and phenylpropanoid metabolism. Primary ammonium assimilation into glutamine/glutamate is carried out by a concerted action of glutamine synthetase (GS) and glutamate synthase (GOGAT). GS1 and GS2 are two distinct isoforms of GS in plants representing glutamine synthetase in the cytosol and chloroplast, respectively. Two molecular species of GOGAT are found, one requiring NADH as reductant (NADH-GOGAT) and the other requiring ferredoxin (Fd-GOGAT). In Arabidopsis, five GS1(*GLN1;1*, *GLN 1;2*, *GLN 1;3*, *GLN 1;4*, *GLN 1;5*), one GS2 (*GLN 2*), two Fd-GOGAT (*GLU1*, *GLU2*) and one NADH-GOGAT (*GLT1*) are encoded in the genome . These GS isoforms may play partially overlapping or distinct roles in specific organs or cell compartments. In the present study, we identified the molecular species of GS and GOGAT responsible for assimilation of NH_4^+ in roots. Real-time PCR analysis showed that levels of *Gln1;2* and *Glt1* mRNA were specifically increased following supply of NH_4^+. The results of promoter-GFP analyses showed *GLT1* gene was mainly expressed in epidermis, cortex and pericycle cells. These results strongly suggest that most of the absorbed NH_4^+ can be assimilated to glutamine and glutamate by the NH_4^+ inducible GLN1;2(GS1)/GLT1(NADH-GOGAT) system at these cells of Arabidopsis root. By contrast, the levels of *GLN1;1* and *GLN1;4* mRNA in root increased within a few days of NH_4^+ deprivation. *GLN1;1* and *GLN1;4* were expressed in the root tip and epidermal cells, respectively. These results suggest that GLN1;1 and GLN1;4 may play roles in assimilation of NH_4^+ concertedly with NH_4^+ transporters inducible by N limitation in Arabidopsis roots.

178 Two Cytochrome P450s Involved in Fruit Elongation in Arabidopsis

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Two Cytochrome P450s Involved in Fruit Elongation in Arabidopsis *Ho Bang Kim[1], Sun Jeong Kim[1], Joon-Hyun Park[2], Sunghwa Choe[1], Chung Sun An[1], Rene Feyereisen[3], Kenneth Feldmann[2]* **[1]School of Biological Sciences, Seoul National University, Seoul 151-742, [2]Ceres Inc., 3007 Malibu Canyon Rd., Malibu, CA 90265, USA, [3]INRA Centre de Recherches d'Antibes, 1382 Route de Biot, 06560 Valbonne, France** Cytochrome P450s are involved in the metabolism of many secondary plant products as well as most phytohormones. However, the function of most P450s remains unknown. In this study, we generated a double mutant for two cytochrome P450 genes, which showed 80 % of amino acid sequence homology and expressed in all tissues investigated. The silique length of the double mutant reduced 40% compared to wild type and single mutants, but the seed number per silique was not changed in the double mutant. In planta leaf senescence was enhanced in the double mutant compared to wild type and single mutants. The transgenic plants overexpressing one of the genes showed defects in silique development and stem growth. GUS activity driven by the gene promoter was strongly detected in fast growing tissues such as young leaf primordia, developing siliques, axillary buds, and floral buds. We will discuss the functional roles of the cytochrome P450 genes in plant growth and development.

179 The Cinnamyl Alcohol Dehydrogenase Family. A SAD Commentary

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The cinnamyl alcohol dehydrogenase (CAD) multigene family in *Arabidopsis* contains nine putative members. Two of these (AtCAD4 and AtCAD5) display significant similarity (81.5 and 82.9%) and identity (75.1 and 76.5%) to the known tobacco lignin-specific CAD (Knight et al., 1992; Halpin et al., 1994), and 3 others (AtCAD6, AtCAD7 and AtCAD8) are 77.3, 77.6 and 78.0% similar and 68.5, 71.4 and 72.1% identical, respectively, to a putative sinapyl alcohol dehydrogenase (SAD) from poplar (Li et al., 2001) claimed to be specific for the formation of sinapyl alcohol (and hence the S component of its lignin). Each cDNA was obtained by PCR amplification, and the individual recombinant proteins were expressed in *E. coli* and purified to apparent homogeneity. Detailed kinetic characterization using *p*-coumaryl, coniferyl, caffeyl, sinapyl and 5-hydroxyconiferyl aldehydes as substrates revealed that none of the 9-membered putative CADs were sinapyl aldehyde specific in contrast to the report of Li et al. (2001). The data in our own studies are again consistent with CAD not being a key (rate-limiting) enzymatic step (Anterola and Lewis, 2002); it instead only fulfills a subsidiary processing role with sufficient enzyme versatility to act on all of the substrates provided in vivo. The 2 CAD homologues of greatest similarity/identity to the tobacco CAD were catalytically most active. Lastly, analysis of the various CAD knockout lines available revealed that none of those examined were able to alter lignin deposition patterns, in agreement with functional redundancy of several of the CAD isoforms.

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Anterola, A. M., Lewis, N. G., 2002. Phytochemistry 61, 221-294.

Halpin, C., Knight, M. E., Foxon, G. A., Campbell, M. M., Boudet, A. M., Boon, J. J., Chabbert, B., Tollier, M.-T., Schuch, W., 1994. Plant J. 6, 339-350.

Knight, M. E., Halpin, C., Schuch, W., 1992. Plant Mol. Biol. 19, 793-801.

Li, L., Cheng, X. F., Leshkevich, J., Umezawa, T., Harding, S. A., Chiang, V. L., 2001. Plant Cell 13, 1567-1585.

180 Interactions between translation factor eIF3 and the COP9 signalosome

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Eukaryotic translation is regulated by signaling pathways impinging on the initiation factor complexes eIF4 and eIF2. In contrast, regulatory roles of the largest initiation factor, eIF3, are poorly understood in any eukaryote. EIF3 consists of at least five core subunits that are conserved from yeast to man as well as accessory subunits with potential regulatory roles. Using Arabidopsis as a model eukaryote, we show that the 40kDa eIF3h subunit, one of the accessory subunits, physically interacts with the COP9 signalosome, a protein complex implicated in the regulation of protein turnover, as well as translation, in response to environmental stimuli. We have characterized the developmental phenotypes of a T-DNA insertion mutant in the eIF3h gene. Signalosome mutants and the eif3h mutant have overlapping phenotypic characteristics, including a postgerminative growth arrest, which is partially ameliorated by exogenous sugar. We hypothesize that a specific subunit of eIF3 can function as a developmental regulator, perhaps in conjunction with the COP9 signalosome.

181 Hexokinase-like proteins in Arabidopsis gemone.

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We previously localized spinach hexokinase 1 to the chloroplast outer envelope membrane (1). However, hexokinase (HxK) activities are associated with different subcellular compartments such as plastids, mitochondria, the Golgi, and cytosol. To assess the subcellular localization of HxK-like proteins in Arabidopsis, we identified all genes encoding HxK-related proteins by BLAST searches. Seven HxK like proteins can be identified in the Arabidopsis genome and HxK proteins can be subdivided into 4 families. Computer based analysis of subcellular localization predicts three different targets in the cell: (i) mitochondria, (ii) plastids, and (iii) cytosolic compartments. We have used GFP-reporter construct to corroborate the computer-predicted subcellular localization. In addition, we started systematic isolation of T-DNA insertion lines for all seven HxK-like proteins in the Arabidopsis genome. We will present our current data on the subcellular localization of group 2 HxK-like proteins and the analysis of the corresponding T-DNA mutants, and on a comparison of the Arabidopsis proteins to the corresponding proteins from spinach and potato.

(1) Wiese A., Groener F., Sonnewald U., Deppner H., Lerchl J., Hebbeker U., Fluegge U.-I., Weber A. Spinach hexokinase 1 is located in the outer envelope membrane of plastids. FEBS Letters 461(1999) 13-18.

182 T-DNA Insertional Mutants: A Strategy to Understand Intron Splicing Factor in *Arabidopsis thaliana*

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Intron splicing involves complex interactions of many proteins in all eukaryotic organisms with critical differences existing between mammalian, yeast, and plant intron recognition. Sequencing of the *Arabidopsis* genome has suggested that some of these differences may be due to duplication and divergence of splicing factor genes that today share only limited identity with their mammalian and yeast counterparts. For a global in vivo analysis of splicing factor (SF) functions in *Arabidopsis* growth and development, we have begun analyzing T-DNA insertions in the 5' promoter region, 5' UTR, or coding exons of a number of SF genes for their effect on the splicing of constitutive and alternatively spliced transcripts. From the SALK T-DNA collection, we have begun characterizing 25 lines for germination rates, phenotypic changes, and RNA splicing patterns. Among the T-DNA lines characterized to date, several of these T-DNA insertion lines have shown a phenotypic change in lines carrying insertions in single copy SF genes with no close homologues in the *Arabidopsis* genome; others with obvious homologues display no phenotype under normal growth conditions.

The two SF genes that we have completed extensive analysis on encode Ser/Arg-rich proteins designated SR45 and SR1/SRp34. T-DNA insertion lines for the SR45 gene have insertions in either the 5' promoter region or the 7th exon region; the T-DNA insertion line for the SR1/SRp34 gene has an insertion in the 5' UTR. Homozygous knockout/knockdown lines for each of these have been identified by using LB/gene-specific primers followed by Southern gel blot analysis, and their corresponding decreases in mRNA abundance have been characterized by RT-PCR gel blot analysis. A series of RT-PCR analyses for alternatively spliced transcripts including SR1/SRp34, U1-70K, SRp30, RSp31, and RuBisCo activase, are being performed to analyze the effects on alternative splicing elicited by reductions in SR1/SRp34 and SR45. Preliminary results suggest that SR1/SRp34 knockouts cause a reduction in SRp30 mRNA accumulation.

183 Detecting the Expression of an NADP+-Malic Enzyme in Arabidopsis thaliana Guard Cells

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NADP+-malic enzyme activity has been implicated in the mechanism of stomatal closure. A search of the Arabidopsis genome revealed the presence of six NADP+-malic enzyme genes. We have analyzed leaf, stem, root, and silique tissue for expression of these genes using RT-PCR in order to begin to identify which of these genes is expressed specifically in the plant guard cells. Results indicate that all of the forms are expressed in all of these tissue types. We are in the process of isolating guard cells to determine which, if any, of the isoforms are expressed in the guard cells. We are also characterizing knockout mutants for two of the six genes and will report preliminary data on this characterization. It is likely that a guard-cell specific isoform of NADP+-malic enzyme would play a role in regulation of plant water loss.

184 An Arabidopsis semi-dwarf mutant is resistant to typhasterol-mediated root growth inhibition

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Brassinosteroids (BRs) are growth-promoting plant steroid hormones. Isolation and characterization of Arabidopsis *dwarf* mutants has been instrumental in characterization of BR biosynthetic enzymes and further validation of the biosynthetic pathways. However, 3 downstream steps including an ultimate one converting castasterone to brassinolide has been left without corresponding *dwarf* mutants. Near complete analysis of all available BR dwarfs indicated that mutants for these steps may not show dwarfism or the mutations are lethal. Thus we employed an alternative method to isolate mutants. Based on assumption that BR intermediates such as typhasterol (TY) and castasterone (CS) should be converted to an end product prior to becoming bioactive BRs, we plated EMS mutant population on TY-containing media and screened for phenotypes displaying unaffected root growth in spite of concentrated TY. After primary screening of >100,000 M2 seeds, 57 lines were identified. The 57 lines were subject to secondary screening to isolate 11 lines possessing more obvious phenotypic difference. One of these mutants was named *semidwarf1* (*sdf1*) for its relatively weak dwarfism as compared to previously described BR *dwarf*. *sdf1* is characterized to have round leaves with slightly reduced stature relative to a wild-type control. Results of various genetic and biochemical analyses will make it clear to define the function of this gene product in BR biosynthetic pathways.

185 Large-scale analysis of albino and pale green mutants using *Ac/Ds* transposon system in *Arabidopsis*.

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We transposed *Dissociation* (*Ds*) elements from 3 start loci on chromosome 5 and 4 loci on chromosome 1 in *Arabidopsis thaliana* and made 9425 *Ds*-tagged lines of F3 plants carrying independent transposition events. To study function of nuclear genes involved in chloroplast development and photosynthesis, we have screened 9425 *Ds*-tagged lines to isolate 87 mutants with albino or pale green (*apg*) phenotypes. Thirty-eight albino phenotypes out of 87 mutants were tagged with *Ds* insertions. We estimated that about 44 % mutants from *Ds* transposon-mutagenized population are tagged with *Ds*. Thus, our *Ds* tagging population is more efficient for gene isolation than T-DNA tagging populations. Eighteen of the *apg* mutants were simple albino, 17 were shown pale green, 3 were white cotyledon and 2 were variegated. BLAST was used to identify the insertion positions in *Arabidopsis* genomic sequence. In 40 *apg* mutants, *Ds* element was inserted into protein-coding genes. Twenty-one genes in the 40 mutants have sequence homology with reported proteins whereas 16 are unknown genes.

186 Carbon export from Arabidopsis chloroplasts at night.

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Maltose is emerging as an important intermediate in the breakdown of leaf starch. We have identified two mutations (*deg262-1* and *deg262-2*) in a gene coding for a novel chloroplastic maltose transporter. Our data indicate that, during the night, carbon from the chloroplasts is exported mainly in the form of maltose in Arabidopsis. The importance of the maltose transporter is emphasised by the severe slow growing and pale green phenotype of the mutants. Interestingly, the transporter bears no homology to any known sugar transporter described so far. However, homologous genes are expressed in other species, indicating that it might be of widespread importance in plants. These results will be discussed in the frame of what is already known about carbon export from chloroplasts.

187 Isolation and characterization of *Arabidopsis* mutants with altered expression of sulfur-responsive genes

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In *Arabidopsis* exposed to sulfur deficiency, activity of sulfate transport and assimilation was increased. Accumulations of mRNAs corresponding to several genes for the sulfur assimilation pathway were also increased. Nitrate application to *Arabidopsis* also upregulate several sulfur assimilation pathway genes. Metabolites such as *O*-aceryl-L-serine (OAS) and glutathione (GSH) are considered to be regulators of genes for sulfur metabolism in response to sulfur or nitrogen nutrition.

In order to conduct genetic analyses of the regulation, we first generated transgenic *Arabidopsis* that carries the green fluorescent protein (GFP) gene under control of a sulfur-responsive promoter (NOB plants). In NOB plants, regulation of gene expression by sulfur can be easily monitored. We used a chimeric promoter comprised of the cauliflower mosaic virus (CaMV) 35S RNA promoter and the β subunit gene of β-conglycinin from soybean. It has been demonstrated that a sulfur responsive promoter region of the β promoter (235 bp) conferred sulfur responsiveness to the CaMV 35S RNA promoter in non-seed tissues. In NOB plants, GFP fluorescence was increased by exposure to sulfur deficiency. NOB also responded to OAS or GSH application in a similar way to endogenous sulfur-responsive genes.

We screened mutants with altered GFP fluorescence from EMS-mutagenized M2 plants of NOB plants. Twenty-five putative mutants were isolated from 40,000 seeds, and we analyzed two of the lines that exhibited higher GFP fluorescence, both under sulfate sufficiency and deficiency, than the original NOB. These lines exhibited 3:1 segregation of the phenotype in F2, suggesting recessive loci are responsible for the phenotype. Fine mapping located the causal genes at upper part of chromosome 5 and 3, respectively. Accumulation of mRNAs corresponding to *Sultr2;2*, *APR1*, and *SAT1*, endogenous sulfur responsive genes, were also higher in both mutants. Both mutants overaccumulated OAS, suggesting that the mutations impaired genes involved in OAS homeostasis.

188 Exploring genomic evidence for a plant-specific nuclear RNA polymerase.

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In searching the Arabidopsis genome for DNA-dependent RNA polymerase second largest subunits, the homologs of eubacterial Beta subunits, we found the expected subunits of pol I, pol II and pol III, but also found an atypical fourth class represented by two genes (MYM9.13 and MRC8.7). Cotton, alfalfa and sorghum genomes also possess genes encoding an atypical second largest subunit, suggesting a conserved function in monocot and dicot plants. Among eukaryotes, the atypical second largest subunits occur only in plants and are most similar to pol II second largest subunits based on sequence similarity. MYM9.13 and MRC8.7 share 92% sequence identity and both are located on Chromosome 3. The expression patterns of the genes have been examined by 5' RACE, RT-PCR and northern blot analysis. MYM9.13 is expressed in all tissues examined and at high level in roots, flower buds and flowers. 5' RACE and RT-PCR experiments failed to detect expression of MRC8.7 in any tissue examined. To estimate the function of the atypical nuclear RNA polymerase subunit, T-DNA insertional mutants of MYM9.13 and MRC8.7 were isolated. In the double-mutant, no expression of atypical subunit expression can be detected, yet plants do not show obvious phenotypic defects, suggesting that the atypical second largest subunits are non- essential. By contrast, T-DNA insertions into the genes encoding the second largest subunits of pol I, II or III are lethal in the homozygous state. Ion-exchange chromatography shows that fractions enriched for the atypical subunit lack RNA polymerase activity. The possibility that the atypical subunit is a repressor of one or more RNA polymerases is being investigated.

189 Examination of 2-Oxoglutarate Dependent Dioxygenases in the Central Flavonoid Biosynthetic Pathway of *Arabidopsis thaliana*

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Flavonoids are a diverse group of natural products found in all higher plants. The flavonols are the most varied and abundant sub-class of flavonoids, and have been associated with a number of essential physiological functions in planta as well as pharmacological activities in animals. Flavonols are represented by two major aglycone structures that result from variations in the flux through the central flavonoid biosynthetic pathway. The enzymatic activities of flavonol synthase (FLS) and flavanone 3- hydroxylase (F3H) are essential in the synthesis of flavonols. FLS and F3H have been demonstrated to be 2-oxoglutarate-dependant dioxygenases based on their requirement for ascorbate, 2-oxoglutarate, and Fe^{2+} for optimum activity levels. Six putative FLS genes (FLS 1-6) have been identified in the Arabidopsis genome while F3H is encoded by a single gene, as are all other enzymes of the central flavonoid pathway. FLS 6 likely represents a pseudogene as it contains a premature stop codon in its sequence, and no corresponding ESTs have been identified. The role of the remaining FLS genes in *Arabidopsis thaliana* is still unknown. We hypothesize that differences in the accumulation of flavonols in different tissues may be the result of differential expression of FLS isozymes with varying substrate specificities. As one approach to testing this hypothesis, the functional isozymes are being expressed in *E.coli* and utilized to develop an enzymatic assay using HPLC as a detection system. An assay for F3H has been achieved, and is currently being utilized to biochemically characterize the recombinant enzyme. The F3H system is also being used as a model on which to generate a reliable FLS assay.

190 Purification and characterization of *Arabidopsis* ribonuclease P, an ancient catalytic RNP complex with potential to serve as an mRNA degradation tool

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Ribonuclease P (RNase P), a ribonucleoprotein (RNP), catalyzes the 5[prime] maturation of tRNAs by endonucleolytic cleavage of precursor tRNAs. This ubiquitous enzyme is essential in all domains of life and has been well characterized in bacteria, yeast and human. RNase P is composed of an RNA subunit and one or more protein subunits depending on the source, ranging from one (bacteria) to nine (yeast) or ten (human) protein subunits. The RNA subunit of bacterial RNase P is a ribozyme on account of its catalytic activity in the absence of its sole protein cofactor, a distinguishing feature between bacterial and eukaryotic RNase P. Why is there a change from a more RNA-based to a more protein-based enzyme in higher evolutionary systems? Could it be that a multi-protein subunit RNase P holoenzyme is necessary for its increased catalytic efficiency or essential to regulate RNase P activity in an environment-responsive or tissue-specific manner? Since we have now identified in plants genomic and EST sequences that are homologous to five of the human RNase P protein subunits, the above questions could be addressed in a more cost-effective manner in plants than other higher eukaryotes. Moreover, characterization of plant RNase P will have practical implications for exploring its potential as a functional genomics tool (see Abstract 524). We have used ion-exchange chromatography to partially purify two isoforms of RNase P from *Arabidopsis* floral buds. These two isoforms are being characterized in terms of their subunit composition. We are using these RNase P preparations to confirm the sequence of the RNase P RNA subunit that we have identified by sequence-homology searches. We are also employing a tandem affinity purification (TAP)-based approach for purifying *Arabidopsis* RNase P and identifying all its protein subunits. In this regard, we have already generated transgenic plants in which the TAP tag was fused to either AtPOP5 or AtRpp29, two putative subunits of *Arabidopsis* RNase P. Results from all these studies will be presented and discussed.

*Contributed equally to this study.

191 Sugars and Phytohormones Regulate Seed Germination and Early Seedling Development via a Complex Signaling "Network"

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Plants undergo profound metabolic and developmental transitions during seed germination and early seedling development. Regulation of these transitions and other processes occurs via a complex signaling "network" that receives input from several chemical signals. These chemical signals have long been known to include abscisic acid, which inhibits seed germination, as well as ethylene and gibberellin, which promote seed germination. Recent work by our lab and others has shown that soluble sugars also affect seed germination. Even low to moderate concentrations of exogenous sugars (e.g. 30 mM glucose) cause a significant delay in the rate of germination of wild-type Arabidopsis. Several lines of evidence also suggest that sugars and phytohormones "interact" in the regulation of early seedling development. For example, several sugar-insensitive (*sis*) mutants of Arabidopsis also exhibit alterations in phytohormone response and/or metabolism. The *sis1* mutant is allelic to the ethylene constitutive response mutant, *ctr1*. The *sis4* mutant is allelic to the abscisic acid deficient mutant, *aba2*, and the *sis5* mutant is allelic to the abscisic acid insensitive mutant, *abi4*. In addition, *sis6* exhibits both sugar and abscisic acid insensitive phenotypes. The *sis6* phenotype is caused by over-expression of a previously uncharacterized gene that encodes a small, PEST-containing protein. Furthermore, the *sis2* mutant displays resistance to the gibberellin biosynthesis inhibitor paclobutrazol, suggesting that *sis2* may be defective in gibberellin response or metabolism. Findings that exogenous glucose greatly exacerbates the negative effects of paclobutrazol on seed germination also suggest "interactions" between sugar and phytohormone response pathways. Interestingly, the *sis3* mutant exhibits a wild-type or near wild-type phenotype in all phytohormone response assays conducted to date, suggesting that some *sis* mutations have a relatively specific effect on sugar response. Possible models for "interactions" between sugar and phytohormone response pathways will be discussed.

192 The Genetics and Biochemistry of Triterpenoid Biosynthesis in Arabidopsis

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Although terpenoids constitute the largest group of natural plant products, the biological roles of few terpenoids are known. The triterpenoids are a family of 30-carbon terpenoids and their derivates, including membrane sterols, the brassinosteroids, and nonsteroidal triterpenoids. Triterpenoid ring systems are constructed from 5-carbon precursors by an enzyme sequence that includes farnesyl pyrophosphate synthase, squalene synthase, squalene epoxidase, oxidosqualene cyclase, and cycloeucalenol isomerase. Arabidopsis has two copies of farnesyl pyrophosphate synthase and squalene synthase, six sequences similar to squalene epoxidase, and thirteen apparent oxidosqualene cyclases. This seeming redundancy suggests that enzyme homologs may have different substrate or product specificity, have altered catalytic abilities, or distinct spatial or temporal expression patterns. We are exploring the triterpenoid biosynthetic repertoire of Arabidopsis using spectroscopic and chromatographic structural determination, and establishing the catalytic properties of the encoded enzymes by heterologous expression in yeast mutants coupled with similar analytical approaches. We are studying the biological roles of triterpenoids by gene expression analyses and phenotypic examination of loss-of-function mutants and plants that heterologously express known yeast homologs. How these alterations affect plant growth, development, and triterpenoid composition will establish specific biological roles for the target genes. Our goal is to provide a comprehensive accounting of triterpenoid skeletons synthesized by Arabidopsis, establish which compounds are derived from each gene product, and determine the spatial and temporal expression patterns of the various biosynthetic genes. (This research is supported by the NSF 2010 program.)

193 Expression and Characterization of Arabidopsis beta-Glucosidases

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β-O-Glucosidases (EC 3.2.1.21) are ubiquitous enzymes that catalyze the hydrolysis of aryl and alkyl β-D-glucosides as well as β-linked oligosaccharides. In plants, these enzymes have been implicated in such processes as defense against pests, phytohormone activation, lignification, and cellulose degradation. The Arabidopsis genome has 46 putative β-glucosidase genes (40 β-O-glucosidases and 6 β-S-glucosidases) which can be divided into 8-10 subfamilies by phylogenetic analysis. We are using a functional genomic approach to discover the biological roles played by each member of the Arabidopsis β-glucosidase gene family. Using the *Pichia pastoris* expression system, we have expressed hydrolases encoded by several members of this family. Four of these enzymes, encoded by At2g44450, At3g18080, At5g44640, and At5g36890, have been purified to homogeneity. To gain insights into the biological roles played by these enzymes, two approaches are being taken. We are determining the aglycone and sugar specificities of each recombinant enzyme *in vitro*. As expected, all four enzymes efficiently hydrolyze *para*-nitrophenyl-β-D-glucoside (PNPG). For At2g44450 and At5g44640, cellobiose and laminarin are the most rapidly hydrolyzed of the natural substrates tested. Interestingly, the hydrolase encoded by At3g18080 cleaves *para*-nitrophenyl-β-D-mannoside twice as fast as PNPG. Metabolic profiling using HPLC is also being employed to identify the natural substrates of each enzyme. The purified At3g18080-encoded hydrolase degrades two compounds in leaf extracts and three in silique extracts; their chemical nature is under investigation.

194 RNase P-mediated disruption of gene expression in Arabidopsis and maize

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Ribonuclease P (RNase P) is a ubiquitous endoribonuclease responsible for the removal of 5[prime]-leader sequences from precursor tRNAs (ptRNAs). Insights derived from studies on the mechanisms of substrate recognition by RNase P has led to a method for degrading any RNA inside living cells. This approach is based on enticing endogenous cellular RNase P into recognizing a specific mRNA as a non-natural substrate, provided that it hybridizes to another RNA sequence called the external guide sequence (EGS). The key requisite is that the EGS should be able to bind a selected target mRNA and generate a sequence- and structure (ptRNA-like)-specific complex, thus rendering the mRNA a substrate for RNase P. Using partially purified RNase P from rice and maize, we demonstrated that plant RNase P can cleave such a non-natural bipartite substrate *in vitro* [Raj et al. (2001) *Plant Physiol.* **125:** 1187]. To evaluate the efficacy of RNase P-mediated degradation of target mRNAs in plants, we designed EGSs against two mRNAs encoding a reporter and an endogenous protein, in a dicot stable expression and a monocot transient expression system, respectively. In the first study, transgenic *Arabidopsis* plants were generated that express the EGS against the green fluorescent protein (GFP) transgene, while the EGS construct against the maize flavanone-3-hydroxylase (F3H) mRNA was introduced transiently into maize cells by micro-particle bombardment. To ensure high-level expression and retain the EGS RNAs inside the nucleus, where RNase P is compartmentalized, these EGS constructs were cloned under the *U3 snRNA* promoter of either *Arabidopsis* or wheat for study in dicot or monocot cells, respectively. In both studies, we observed down-regulation of the targeted proteins. Design of disabled mutant EGSs, which retain regions complementary to the target mRNAs but would not generate an RNase P substrate upon binding to their respective target mRNAs, is in progress to confirm that the observed down-regulation is due to RNase P-mediated cleavage and not antisense effects.

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195 *Dct* is deficient in the plastidic glutamate/malate translocator

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The Arabidopsis mutant *dct* is one of the classic mutants in the photorespiratory pathway. It requires high CO_2 levels for survival. Physiological and biochemical characterization of *dct* indicated that *dct* is deficient in the transport of dicarboxylates across the chloroplast envelope membrane (1). However, the defective gene in *dct* has not been identified at the molecular level and the gene product has not been characterized.

Here, we report on the molecular characterization of the defective gene in *dct*, on the complementation of the mutant phenotype with a wild type cDNA, and on the functional characterization of the gene product in a recombinant reconstituted system.

Dct is deficient in a metabolite transporter of the plastid inner envelope membrane, DiT2. DiT2 is a glutamate/malate translocator (see (2) for a recent review on plastidic dicarboxylate transport). It is exporting the end product of ammonia assimilation, glutamate, from the plastid stroma to the cytosol. Glutamate is required in the photorespiratory pathway for the transaminiation of glyoxylate to glycine in the peroxisomes. A deficiency in glutamate export from plastids interrupts the glutamate supply to peroxisomes. Hence, glyoxylate can no longer be converted to glycine. Consequently, the toxic metabolites glyoxylate and glycolate accumulate. This leads to an inhibition of carbon dioxide assimilation of by the Calvin cycle. Moreover, the recycling of the oxygenation product of RubisCO (glycolate) ceases.

Our results demonstrate that the plastidic glutamate/malate translocator DiT2 is absolutely required for plant growth in ambient air.

(1) Somerville SC and Ogren WL (1983) An *Arabidopsis thaliana* mutant deficient in chloroplast dicraboxylate transport. PNAS USA 80: 1290-1294.
(2) Weber A and Fluegge, UI (2002) Interaction of cytosolic and plastidic nitrogen metabolism in plants. J Exp Bot 53: 865-874.

196 Commonalities in the regulation of a secondary metabolite-specific promoter in *A. thaliana* and *N. tabacum*

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The regulation of a promoter for a gene in a highly regulated secondary pathway from tobacco was studied in *Arabidopsis* to address the degree of conservation in signaling mechanisms and to identify components that might be held in common. Putrescine *N*-methyltransferase (PMT) catalyzes the first committed step in the biosynthesis of pyrrolinium ring-containing alkaloids. *PMT* gene expression is primarily restricted to root tissue in Solanaceae plants. *PMT* gene expression is upregulated in response to leaf wounding via JA-dependent pathways and its negatively regulated by auxin. GUS (β-glucuronidase) promoter fusions of the *PMT* gene from *N. tabacum* were introduced into both tobacco and *Arabidopsis* and the resulting plants were examined. In *N. tabacum*, upregulation of the *PMT* gene promoter was observed in roots upon treatment with JA while treatments with auxin, SA and H_2O_2 and irradiation of roots with light strongly represses the promoter activity. Similar responses have been observed in *Arabidopsis* and we will take advantage of the genetic resources available for *Arabidopsis* to help decipher the mechanism of these signaling molecules. For example, in *A. thaliana*, treatment with ROS (reactive oxygen species) similarly represses the activity of the *PMT* promoter. Light has been shown to cause an increase in the production of ROS thereby triggering various changes in the gene expression. Reports suggesting ROS as an intermediate in plant hormone signaling may also indicate the involvement of ROS in auxin-mediated repression. Therefore, we are currently investigating the role of auxin in relation to ROS with the help of *Arabidopsis axr* (auxin resistant) mutants. The diverse regulation apparently in control of the alkaloidal pathway in tobacco may only be the result of specialization of input signals. Signaling components still active in *Arabidopsis*, such as those that function as downstream intermediates, may indicate signals and mechanisms that are in common and therefore conserved between the two species.

197 The In Planta Role of the Arabidopsis Pht1;1 and Pht1;4 in Pi Nutrition

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Using a reverse genetics approach, we isolated Arabidopsis T-DNA/transposon insertion mutants defective in the *Pht1;1* (*pht1;1-1* and *pht1;1-2*) and *Pht1;4* (*pht1;4-1* and *pht1;4-2*) genes encoding high-affinity phosphate (Pi) transporters. These single mutants displayed reduced rates of Pi uptake by roots, supporting previous suggestions that the Pht1;1 and Pht1;4 proteins mediate Pi acquisition from soils. The Pi uptake rate of a double mutant *pht1;1Δ4Δ*, generated by crossing *pht1;1-2* to *pht1;4-1*, was much more reduced than each of the single mutants, within a range between 20.9 and 44.3 % of the wild type regardless of the Pi growth condition. The Pi uptake rate of the double mutant was increased by Pi-starvation, indicating that Pi-starvation-inducible Pi transporter activities are still present in the double mutant background. The double mutant displayed several visible phenotypes under certain Pi conditions, all related with less Pi uptake capability. Interestingly, arsenate tolerance was dramatically increased by the double mutant, providing evidence that arsenate uptake by plants also is mediated by the Pht1 type Pi transporters. Overall, our results indicate that the Pht1;1 and Pht1;4 play a major role in mediating Pi acquisition by roots under a wide range of Pi conditions.

198 AGD2 and ALD1: Related enzymes with divergent roles in development and defense

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The *aberrant growth and death 2* (*agd2*) mutant of Arabidopsis has increased resistance to a bacterial pathogen, elevated salicylic acid (SA) levels, spontaneous cell death, and enlarged cells in leaves. The *agd2* phenotype is caused by mutation of the *AGD2* gene encoding a protein homologous to aminotransferases. Arabidopsis has one homolog of *AGD2*, which we named *ALD1* (*AGD2 like-defense response protein* 1). The recombinant AGD2 and ALD1 enzymes have overlapping broad substrate specificities with lysine, alanine, arginine, methionine, glutamine, asparagine and leucine as amino donors, and the highest activity with lysine. *AGD2* was suppressed by dark treatment whereas *ALD1* was induced by a SA agonist treatment or pathogen infection. *ALD1* is highly expressed in *agd2* and other cell death mutants including *acd5* and *acd6*, but not in wild-type plants. T-DNA knockout mutants of AGD2 and ALD1 showed that AGD2 is essential for seed development whereas ALD1 functions to confer disease resistance to *Pseudomonas syringae*. We suggest that AGD2 synthesizes an important growth factor while ALD1 generates a related amino acid-driven signal important for activating defense signaling.

199 Identification and characterization of dehydrodolichyl diphosphate synthase genes in *Arabidopsis thaliana*

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In higher plants, a variety of *Z,E*-mixed polyisoprenoid alcohols including polyprenol and dolichol have been detected. The chain length distributions of *Z,E*-mixed polyisoprenoid alcohols are different among each tissues and subcellular fractions. Dolichol has been known to play an important role as a glycosyl carrier lipid in the biosynthesis of glycoproteins and glycosylphosphatidylinositol-anchored proteins. On the other hand, physiological role of polyprenol is hardly elucidated. In order to understand the physiological roles of *Z,E*-mixed polyisoprenoid alcohol in *Arabidopsis thaliana*, we cloned and characterized genes encoding Dehydrodolichyl diphosphate (DedolPP) synthase, which catalyzes *cis*-prenyl chain elongation to produce the polyprenyl backbone of polyprenol and dolichol.

A cDNA encoding DedolPP synthase in Arabidopsis has been reported by two groups (Oh et al, 2000, Cunillera et al, 2000). In Arabidopsis genomic sequences, we found additional 8 genes that have high homology to DedolPP synthase genes isolated from Arabidopsis and yeast. The deduced amino acid sequence of these putative DedolPP synthase genes has high homology to each other (28-90% identity) and shows the presence of all five conserved regions among *cis*-prenyltransferases isolated from various organisms. To analyze the expression pattern of DedolPP synthase genes in various tissues, we performed RT-PCR using total RNA prepared from root, leaf, stem, flower and silique as a template. The expression of two clones could not detected by RT-PCR, suggesting that they might be pseudo genes or expressed in some limited regions at low level. The expression of the other genes could be detected in all of the tissues we checked. However, the variation of expression level among the tissues was different in each clone. According to the genomic sequences and the expression patterns, we have cloned 6 cDNAs by RT-PCR using total RNA as a template. We are currently analyzing the function of gene products and knockout mutants of these genes.

200 Website Updates for NSF2010: Functional Genomics of Arabidopsis P450s and Identification of Some Unusual P450 Transcripts

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As a part of our NSF2010 project, http://arabidopsis-p450.biotec.uiuc.edu has been developed and updated to include P450 family information with gene maps and structure information derived from TAIR and TIGR annotations, nucleotide alignments with all available full-length cDNAs as well as upstream and downstream flanking sequences. Interactive BLAST alignments of the highest identity EST and cDNA sequences and web pages for each P450 EST and cDNA show their sequences and all associated database information. Compilation of this information has allowed us to reanalyze all of the P450 gene models available in TAIR and TIGR and reannotate several models that suffered from incorrectly predicted translation start sites, introns and/or polyadenylation sites. Gene models have been verified by alignments with full-length cDNAs and ESTs and by some RT-PCR analysis. This curation has resulted in identification of new start sites in the CYP79F1 gene, intron positions in the CYP51A2 gene, and transcription termination sites in the CYP72A11 gene. This analysis has uncovered several alternatively included/excluded introns in the CYP71B2 and CYP71B29 genes where intron inclusion and intron excision, respectively, resulted in prematurely truncated P450s. Curation of the CYP79C subfamily has resulted in deletion of one P450 pseudogene from this 4-member subfamily. The analysis also identified several unusually long transcripts derived from tandem P450 genes (CYP705A15/CYP705A16, CYP71B34/CYP71B35) containing two sets of P450 coding sequences and another (CYP71A11/CYP71A12P) containing one P450 coding sequence and an incomplete P450 pseudogene. Another unusually long transcript contains the CYP97C1 coding sequence and the downstream coding sequence (CYP97C1/OMT) suggesting the possibility of a polycistronic transcript for these two loci.

201 Overlapping activities of carotenoid hydroxylases in Arabidopsis

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Lutein and zeaxanthin are dihydroxy xanthophylls that perform critical roles in photosystem structure and function and are derived from their corresponding carotene precursors by the action of β- and ε-ring carotenoid hydroxylases. Two genes encoding β-ring hydroxylases (β-hydroxylase 1 and 2) have been cloned from Arabidopsis and are highly active towards β-rings but only weakly active toward ε-rings. A third gene encoding a distinct activity required for ε-ring hydroxylation (the product of the *LUT1* locus in Arabidopsis) has been defined genetically, but has not yet been cloned. In order to address the individual and overlapping functions of the three Arabidopsis carotenoid hydroxylase activities *in vivo*, T-DNA knockout mutants corresponding to β-hydroxylase 1 and 2 (*b1* and *b2*, respectively) were isolated and all possible hydroxylase mutant combinations were generated together with *lut1*. Carotenoid composition in leaf and seed tissue, expression of β-hydroxylases and lycopene cyclases, NPQ induction and amplitude were analyzed in these mutants. Overall, the hydroxylase mutants provide insight into the unexpected overlapping activity of carotenoid hydroxylases *in vivo*.

202 Effects of Knock-outs in the L3 Ribosomal Protein Gene Family

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Ribosomes are essential organelles composed of ribosomal RNA (rRNA) and ribosomal proteins (rProteins). Ribosomes are responsible for the production of proteins based on the information found in mRNA. It has been shown that rRNA catalyzes the reactions that are directly responsible for protein synthesis; however, rProteins may play accessory roles in protein synthesis. However, some proteins are known to play roles in translation. While it appears that all ribosomes have the same proteins, in at least some organisms it is apparent that different family members exist for some if not all rProteins. This raises the interesting possibility of specialized ribosomes.

Stochiometric amounts of each rProtein must be present for efficient assembly of ribosomes. Therefore it follows that rProtein gene regulation is necessary for a healthy organism. In E. coli each rProtein is encoded by a single gene. In mammals there are small gene families for each rProtein, however it appears that only one member is functional. In Arabidopsis, small gene families with two or three functional members, encode most proteins. The deduced amino acid sequence of each member in plants varies from identical to 74% identity.

We have characterized two members of a ribosomal protein gene family (RPL3A and RPL3B) from Arabidopsis. These two genes share approximately 86% amino acid identity. Based on Western analysis, the L3A protein appears to be essentially constitutively expressed at high levels in all tissues. L3B is more abundant in root than shoot. L3B mRNA has several unusual features. Its 5[prime] UTR is very short, but is otherwise normal and, it has a very short polyA tail. We have some evidence that mRNA processing may be involved in the regulation of L3B levels.

T-DNA insertion mutants from the SALK collection have been identified for L3A. The mutation appears lethal when homozygous, and the hemizygote shows slower growth compared to wild type. In addition, the insertion is transmitted to the F2 generation from hemizygote parents in 1:1 ratios instead of the expected 2:1.

T-DNA insertions have also been found in the 3[prime] and 5[prime]UTRs of L3B; however, these have not shown a visible phenotype.

203 Lipid Metabolism and Chloroplast Biogenesis are Impaired by Glucose

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Soluble sugar levels must be closely regulated in germinating seeds to ensure an adequate supply of energy and building materials for the developing seedling. Although numerous studies have shown that sugars inhibit starch mobilization by germinating cereal seeds, comparatively little research has been done on the effects of sugars on lipid breakdown by oilseed plants, such as Arabidopsis. Results presented here indicate that mobilization of storage lipid by germinating Arabidopsis seeds is greatly inhibited in the presence of exogenous glucose or mannose, but not by equi-molar 3-*O*-methylglucose or sorbitol. The sugar and abscisic acid insensitive mutant, *abi4-101/sis5-1*, is resistant to glucose inhibition of seed storage lipid mobilization. Wild-type seedlings become insensitive to glucose inhibition of storage lipid breakdown within 3 days of the start of imbibition, suggesting that completion of some metabolic or developmental transition results in loss of sensitivity to the effect of sugars on lipid breakdown. High concentrations of exogenous sugars also inhibit greening of wild-type Arabidopsis seedlings. To determine whether growth on glucose impairs chloroplast biogenesis, the levels of hexadecatrienoic (16:3) fatty acid, a chloroplast-specific fatty acid, were measured in Arabidopsis seedlings grown on media containing different concentrations of glucose. These experiments indicate that moderate concentrations of glucose delay accumulation of 16:3. The effects of glucose on 16:3 levels are not solely due to osmotic stress, as equi-molar and even twice equi-molar concentrations of sorbitol do not exert comparable effects. In addition, electron microscopy studies reveal that seedlings grown on high concentrations of glucose lack identifiable chloroplasts.

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204 Exploring the roles of flavonol synthase isoforms in flavonoid metabolism

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Flavonols perform diverse functions in plants as UV sunscreens, defensive molecules, and regulators of hormone transport. Proportionate levels of the two main flavonols in *Arabidopsis*, quercetin and kaempferol, can change with tissue and developmental status; however the biochemical basis for this variation remains to be elucidated. In the flavonoid metabolic pathway, flavonol synthase (FLS), which converts dihydroflavonols to flavonols, is the only enzyme that is encoded by more than one gene. Previous research indicated that FLS isoforms may be specific for a given dihydroflavonol subclass, suggesting that FLS isoforms may regulate synthesis of specific flavonols. We are testing the hypothesis that FLS isoforms are tissue-specific and are differentially expressed during development and in response to environmental signals. Promoter:GUS constructs were engineered for five FLS isoforms, which were transformed into *Arabidopsis*. Promoter expression is being followed through plant development.

205 Genes from Arabidopsis involved in Iron-Sulfur cluster biogenesis

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Proteins that use iron-sulfur [Fe-S] clusters as prosthetic groups contribute key activities in primary metabolism, hormone biosynthesis, energy production, and the stress response in plants. However very little is known about the assembly of [Fe-S] clusters in plants, despite the progress that has been made toward understanding this process in bacteria, yeast, and mammals. Arabidopsis thaliana contains two counterparts of the Azotobacter vinelandii IscS gene (designated AtNFS1 and AtNFS2) and three counterparts of IscU (AtISU1, AtISU2, and AtISU3). The significance of the redundancy of these genes in Arabidopsis is unknown. Transgenic plants have been generated to determine the biological function of these genes using suppression studies. Further, transgenic plants have been generated with the goal of defining the subcellular localization of the proteins using fusions of AtNSF1 and AtISU proteins with GFP. The AtNSF1 and AtISU1 genes have been expressed in Escherichia coli and in vitro biochemical characterization of the recombinant proteins has confirmed the ability of the gene products to assemble [Fe-S] clusters. Promoter fusions are being constructed to determine if transcription of these genes is inducible or involved in [Fe-S] assembly processes that are tissue or organelle-specific. Gene-specific antibodies are being developed to enable analysis of expression at the protein level.

206 Characterisation of a small multigene family, encoding group l glycosyltransferases involved with the biosynthesis of phenylpropanoid glucoside esters in arabidopsis

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Recent bioinformatic approaches have identified a 107-member, class 1 UDP-glycosyltransferase family in arabidopsis. As a class, these enzymes act to add glucose moieties from UDP-glucose to a wide range of small metabolites via both ester and glucosidic linkages. These reactions are generally considered to produce high-energy metabolic intermediates or storage forms of the aglycone respecively. A number of factors such as wide ranging substrate specificities and product formation (*in vitro*) has made assignment of function, based on sequence similarities, very difficult. In order to unambiguously assign function to members of this gene family, detailed multi disciplinary investigation of the *in vivo* biochemistry and physiology is required. In an attempt to define the *in vivo* role of members of this family we are currently focusing on a small 4-member subfamily from group L that we have shown to be able to catalyse the formation of glucose esters of the cinnamic derived acids, coumaric, ferulic and sinapic acid. One member of this subfamily has been shown to catalyse the formation of sinapoyl glucose from sinapic acid. The formation of this compound is proposed to be a high-energy intermediate involved in the formation of sinapoyl malate and sinapoyl choline in leaves and seeds respectively. Our current work has focused on the identification of the expression patterns of these 4 genes and the characterisation of both CaMV 35S driven over-expression, PTGS derived “knock-down” transgenic lines and T-DNA insertional mutants for all 4 genes. Data will be presented showing both the temporal and spatial localisation of all 4 genes in wild type arabidopsis and the preliminary results of the effects of over-expression and knock-down/out of these 4 glycosyltransferases.

207 Anionic lipids are essential for chloroplast structure and function

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The chloroplast of plant cells contains two major anionic lipids, Sulfoquinovosyl diaylglycerol (SQDG) and phosphatidyl diacylglycerol (PG). Previously, it was proposed that SQDG can substitute PG to maintain a constant amount of anionic lipids during phosphate deprivation. Here we report the generation of an Arabidosis double mutant deficient in SQDG and reduced the amount of PG. Lipid analysis showed that the total content of anionic lipids was reduced in the double mutant. The double mutant had pale yellow cotyledons and leaves. The growth of the double mutants was compromised, although it was capable of autotrophic growth. In addition, the photosynthetic capacity was impaired in the double mutant. Furthermore, the double mutant had altered leaf structure and a reduced number of mesophyll cells. The ultrastructure of chloroplast was also changed in the double mutant. These results suggested that the constant content of anionic lipids is required to maintain plant growth, chloroplast structure and function.

208 Screen of *Arabidopsis* progesterone resistant mutants

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Progesterone is one of the typical mammalian steroid hormones, whose functions are mediated primarily via nuclear receptor proteins in animal cells. While plants are not known to encode similar nuclear receptors, we found that 30 μM progesterone strongly inhibits *Arabidopsis* seedling growth on synthetic agar media during our work related to the production of nuclear receptor agonists in plants. On the other hand, the same or higher concentrations of estrogen, betamethasone and campesterol, compounds which have structures similar to progesterone, showed little or no growth inhibition, suggesting specific targets in the plant cell. *Arabidopsis det1-1* and *det2-1* mutants, which are deficient in sterol biosynthesis, showed similar growth responses to progesterone as the wild type. *Det2-1* is defective in 5 α-reductase, which has been shown to use progesterone as a substrate. The inhibiting effect of progesterone in the wild type therefore is unlikely to be due to the product of the 5α-reductase enzyme using progesterone as the substrate, a result suggesting interactions with other cellular components. To begin to elucidate how these specific growth-inhibiting effects of progesterone occur, we screened T-DNA knockout populations for mutants that show tolerance to progesterone. Out of 10,000 mutant lines screened to date, more than 200 putative progesterone-tolerant mutants have been obtained. Progress in secondary screening and characterization of the primary mutants will be presented.

209 Characterization of AtTSC13, an Arabidopsis homologue of the Saccharomyces cerevisiae enoyl-reductase TSC13

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The membrane-bound fatty acid elongation enzyme system consists of four components that catalyze four successive enzymatic reactions: condensation, reduction, dehydration and a second reduction required for very long chain fatty acid (VLCFA; 18C or longer) biosynthesis. In plants, while the basic biochemical steps for the VLCFA elongation are believed to be conserved, only a few proteins that catalyse the condensation reaction have been identified. To further dissect the plant fatty acid elongation enzyme system, the function and sub-cellular location of AtTSC13, a single homologue of the yeast enoyl-reductase in Arabidopsis, that catalyzes the second reduction reaction in the elongation of VLCFAs are being investigated. Transgenic lines have been generated with RNAi constructs containing the AtTSC13 gene under CER6, FAE1 and 35S promoters that are expected to suppress the expression of the protein in the epidermis, the seed and the entire plant, respectively. The effect of AtTSC13 suppression on the cuticular wax profile and seed lipid composition will be presented. In addition, tissue and cellular localization of the AtTSC13 fused with GFP at either the amino or carboxyl terminus will be determined.

210 Investigating several CLE genes as possible extracellular ligands

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The *Arabidopsis* genome contains over 600 receptor-like kinases (RLKs), so named because each one has an extracellular domain connected by a single-pass transmembrane domain to an intracellular kinase moiety. Specific functions have been assigned to many RLKs, including diverse roles ranging from development to pathogen defense. Only a handful of ligands for RLKs have been identified, and at present, only two endogenous and peptide-based ligands from *Arabidopsis* have been characterized: CLAVATA3 (CLV3) and phytosulfokine. CLV3 is found in the stem cells in the central zone of the meristem and is known to regulate cell proliferation via binding to the CLAVATA1 (CLV1) receptor. *CLV3* belongs to a family of 25 *CLV3*/*ESR* (*CLE*) genes, all of which encode putative small secreted or membrane-bound proteins with a 15-residue region of homology in the C-terminus (ref. 1). Three proteins — CLE11, CLE12, and CLE13 — show strong similarity to each other and to CLV3 within this small domain. All but one of the 25 *CLE* genes is expressed, as determined by RT-PCR (ref. 2).

To uncover possible functions for a select group of CLE proteins (CLE11-13 and 16-18), we have begun to employ reverse genetics approaches, including overexpression. High levels of CLE16, 17, and 18 have no obvious effect on the meristem (although *35S::CLE16* and *35S::CLE17* produce leaves which are narrower than normal). However, *35S::CLE12* bears a striking resemblance to *35S::CLV3* (ref. 3), with early termination of the primary shoot meristem, a distorted, bushy appearance, and flowers which lack a gynoecium and are missing stamens. This result hints at the possibility that CLE12, at high levels, is capable of signaling through CLV1. Ongoing experiments will determine whether the *35S::CLE12* phenotype is epistatic to that of *clv1* or *clv3* lof mutants. In addition, expressing *CLE12* via the *CLV3* promoter, in a *clv3* background, will provide a more subtle picture of its signaling potency. Finally, we are in the process of analyzing the *35S::CLE11* and *35S::CLE13* phenotypes.

(1)Cock J and McCormick S. Plant Physiology 126: 939 (2001)
(2)Sharma V, Ramirez J, and Fletcher J. Plant Molecular Biology 51: 415(2003)
(3)Brand U et al. Science 289: 617 (2000)

211 A dominant-negative approach uncovers a role for *KNAT3* and *KNAT4* in plant development

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The *KNAT3* and *KNAT4* genes belong to the Class II family of knotted-like homeobox transcription factors in *Arabiopsis*. The role of some Class I knotted-like genes such as *SHOOTMERISTEMLESS*, *KNAT1/BREVIPEDICELLUS* and *KNAT2* in meristem development and plant architecture has been established. However, no function has been assigned to date to Class II *KNAT* genes based on mutational analysis. We have used CHRIS (**Ch**imeric **R**epressor **I**nterference **S**ystem) as an approach to further understand *KNAT3* and *KNAT4* function. This technique transforms transcriptional activators into repressors by creating a dominant-negative translational fusion between the engrailed (en) repressor domain from *Drosophila* and the open reading frame of a specific transcription factor. This approach has proven successful in producing phenocopies of loss-of-function mutants and overcoming genetic redundancy. The phenotype of the *en-KNAT3* and *en-KNAT4* transgenic plants suggests a role for both genes in many aspects of plant development, including hypocotyl elongation and leaf and petiole development. The KNAT3 and KNAT4 proteins are capable of forming heterodimers in vitro, and we propose that both proteins act redundantly to control cell division or expansion.

212 The *DORNROESCHEN/ENHANCER OF SHOOT REGENERATION1* gene of *Arabidopsis* controls meristem cell fate and lateral organ development: isolation of potential interaction partners.

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The *DORNROESCHEN* (*DRN*) gene belongs to the ERF-type (Ethylene Response Factor) family of transcription factors. An overexpressing mutant of *DORNROESCHEN* (*drn-D*) shows premature arrest of shoot meristem activity and the formation of radialized lateral organs. This demonstrates two functions of the gene product, in controlling meristem development and in influencing lateral organ formation. Work from another group has also implicated a function for the gene in shoot cytokinin responses. We are further analysing the function of this gene by studying deletions of the *DRN* promoter fused to a GUS reporter gene to identify promoter regulatory elements. We have also performed a two hybrid screen and isolated several potential interacting partners. Amongst these candidate proteins is a homeodomain protein, a bHLH protein and an *Arabidopsis* homologue of a *Nicotiana* protein known to be involved in cytokinin and auxin response. Progress on the characterisation of these partners will be presented.

213 Functional Analysis of *AN* Gene that Regulates Leaf Width

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The morphology of *Arabidopsis* leaves is regulated by the orientation of cell division and cell elongation. The *ANGUSTIFOLIA* (*AN*) regulates the polar elongation of leaf cells in the leaf-width direction (Tsuge *et al*., 1996). Our cloning of the *AN* gene revealed that it encodes a homolog of human CtBP (carboxy-terminal binding protein), which is known as a transcriptional co-repressor (Kim *et al*., 2002). AN is the only member of CtBP family in *Arabidopsis*. AN has 2-hydroacid dehydrogenase domain which is shared with other CtBPs, but has also some AN-specific domains such as LXCXE domain which is related to RB binding, PEST domain which is thought to be responsible for degradation, and nuclear localization signal (NLS). *Drosophila* CtBP interacts with itself, thus, we examined the ability for AN for self-association and for interaction with truncated forms of AN in yeast two-hybrid system. The result clearly showed the AN self-associates by its C-terminal region. an-1 has a mutation point in the C-terminal region of the AN. On the other and, the human CtBP was identified as a protein that binds with the C-terminus of adenovirus E1a, and we tested the interaction of AN with E1a. We did not obtain any evidence on the interaction in the Yeast two-hybrid system. Besides with the protein-level features, we found a curious nature of the AN in terms of the dose-dependency of the *AN* mRNA on the leaf morphogenesis. In a complementation test, the *an-1* mutant phenotype was reversed by *AN* cDNA, but the efficiency of restoration of a wild-type phenotype was low. Quantitative analysis by RT-PCR revealed that the accumulation of abnormally large amounts of *AN* mRNA was responsible for the *an-1* phenotype. The relationship between the level of expression of the *AN* transcript and the phenotype of leaves resembled a mathematical sine curve. Thus, we propose that control of leaf width by the *AN* gene be designable sinusoidal regulation.

214 Receptor kinase functional overlap

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The CLAVATA1 (CLV1) receptor kinase controls stem cell number and differentiation at the Arabidopsis shoot and flower meristems. Other components of the CLV1 signaling pathway include the secreted putative ligand CLV3, and a receptor-like protein CLV2. We report evidence indicating that all intermediate and strong *clv1* alleles are dominant negative, and likely interfere with the activity of unknown receptor kinase(s) with functional overlap with CLV1. *clv1* dominant-negative alleles show major differences from dominant-negative alleles characterized to date in animal receptor kinase signaling systems, including the lack of a dominant-negative effect of kinase domain truncation and the ability of missense mutations in the extracellular domain to act in a dominant-negative fashion. We analyzed chimeric receptor kinases by fusing CLV1 and BRI1 coding sequences and expressing these in *clv1* null backgrounds. Constructs containing the CLV1 extracellular domain and the BRI1 kinase domain were strongly dominant-negative in the regulation of meristem development. Furthermore, we show that *CLV1* expressed within the pedicel can partially replace the function of the ERECTA receptor kinase. We propose the presence of multiple receptors regulating meristem development in a functionally related manner, whose interactions are driven by the extracellular domains and whose activation requires the kinase domain.

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215 The overexpression of a novel homeobox gene WHO gives rise to overall malfunction of Arabidopsis meristem maintenance and organ development

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We have isolated a novel homeodomain transcription factor gene Wuschel-like Homeodomain Overexpressing (WHO) in Arabidopsis, which shares a high degree of homology to WUSCHEL (WUS), a transcription factor involved in specifying stem cell fate in shoot and floral meristems. WHO encodes 182 amino acids with 2 exons. There are 4 single nucleotide polymorphisms found in Lansberg erecta versus ecotype Columbia, with no amino acid change though. The knock out mutant shows normal growth and development, however an overexpression mutant exhibits a severely stunted phenotype, and defects are observable in shoot, floral and root meristems. The mutant has over-sprouting shoots, malformed leaves, strongly-stunted roots, delayed bolting and flowering, as well as aberrant floral organs. Moreover, callus and somatic embryos form on the petioles and hypocotyls of the mutant plants without any external plant hormone treatment. Morphological studies showed that the mutant root has a radial organization defect, with 7 cortical and endodermal cell files, and nodule-shape lateral roots. Overexpression of WHO upregulates CLAVATA3 (CLV3) and SHOOTMERISTEMLESS (STM) expression, but not WUS and CLAVATA1 (CLV1). Our results indicate that this new homeobox gene is redundant with other homeobox genes, however its misexpression disrupts normal overall development in Arabidopsis, suggesting WHO play an important role in maintaining meristem cell identity and organ formation.

216 Cell division, cell expansion and leaf morphogenesis in *Arabidopsis*

Joyce Gardner and Sue Bougourd

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The relationship between cell division, growth and morphogenesis in plants is the subject of considerable debate. In this project, we are investigating the role of cell division during leaf morphogenesis in *Arabidopsis*. Leaf primordia are initiated on the flanks of the shoot apical meristem (SAM), a highly specialised structure that acts as a continuous site of organ formation. A GAL4 transactivation system is used to misexpress cell cycle regulators in specific domains of the SAM and leaves. Preliminary results indicate that misexpression of the dominant-negative mutant of *CDC2aAt* (*cdc2aAt.DN*) specifically in the epidermal L1 layer gives rise to plants with distinctly serrated leaves. Alterations in leaf size and shape will be related to the effects of misexpression on the numbers and sizes of cells, and the role of cell division in determining plant organ shape will be discussed.

217 Specific Myb-bHLH interactions regulate specific TTG-dependent pathways

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Combinatorial interactions among transcription factors are often necessary for proper gene regulation. Such interactions may confer biological specificity to transcription factors with very similar DNA binding motifs or regulate levels of transcriptional activity. In Arabidopsis, the different TTG-dependent epidermal fate pathways appear to require multicomponent transcriptional complexes, presumably with specific combinations of Myb-bHLH protein interactions specifying which pathway is realized. This hypothesis is supported by yeast-2-hybrid experiments, mutant phenotype analysis, and over-expression studies that show, for example, that anthocyanin specific Mybs (PAP1 and 2) interact with partially redundant bHLHs (GL3 and EGL) in the pigment pathway, while the trichome specific Myb (GL1) can interact with these bHLHs to initiate trichomes. Furthermore, these studies also reveal that the bHLHs have some functional specificity such that EGL, for example, has a greater influence over GL3 in specifying anthocyanin production, while GL3 appears to play a greater role over EGL in specifying the trichome pathway.

218 Transcriptome of Arabidopsis Leaf Senescence

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Leaf senescence is a complex developmental process that designed to recycle nutrients. To unravel molecular regulatory mechanisms underlying this process, it is necessary to fully understand the biochemical components and the entire transcriptome associated with leaf senescence. An Arabidopsis leaf senescence cDNA library with approximately 104 recombinant clones was arrayed in 384-well plates, and were subsequently sequenced using large-scale single-pass sequencing. This leaf senescence-specific cDNA library was constructed using mRNAs isolated from Arabidopsis senescent leaves. After removal of low quality sequences, rRNA and tRNA sequences, and a few non-Arabidopsis genomic sequences, 6193 expressed sequence tags (ESTs) were obtained which represent 2491 unique genes. Among them are 134 genes encoding transcription factors and 182 genes whose products are components of signal transduction pathways such as the mitogen-activated protein kinase (MAPK) cascades. There are 116 genes that are predicted to be involved in protein turnover; these include 75 genes associated with ubiquitin-proteasome pathway and 35 proteinases. Consistent with nutrient recycling during leaf senescence, there exist many ESTs/genes that encode a variety of transporters for substances such as ions, amino acids and sugars. In addition, this study revealed 174 novel ESTs (most of them are singletons) and 46 novel transcribed units that have not been annotated in the Arabidopsis genome. Functions of approximately one third of the 2491 genes are predicted to be unknown. The genes are distributed evenly on the 5 chromosomes.

219 *CUP-SHAPED COTYLEDON3*; a novel NAC box gene involved in embryonic and axillary meristem ontogeny together with *CUC1* and *CUC2*.

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Nara Institute of Science and Technology

The shoot apical meristem (SAM) develops during embryogenesis and continuously generates the above ground parts after germination. *CUC1* (*CUP-SHAPED COTYLEDON1*) and *CUC2* encoding NAC domain proteins respectively, are redundantly involved in SAM formation and cotyledon separation during embryogenesis. Although each *cuc1* or *cuc2* single mutant results in almost no obvious phenotype, *cuc1 cuc2* double mutant has one cup-shaped cotyledon and lacks in an embryonic SAM. To identify genes involved in these processes together with *CUC2*, we undertook a screen for second-site mutations that enhanced the phenotypes of *cuc2*. 14 enhancer mutants were isolated from 3,500 M2 lines treated with EMS-mutagenesis. 7 of them were novel *cuc1* alleles. One enhancer gene was mapped close to nga692 marker on chromosome 1. This region included a novel NAC box gene which belongs to the CUC subfamily. A single base pair mutation which affected the splicing of transcripts was found in the gene. Additionally, the other 3 lines had the mutations in the same gene at the different positions. Then, we renamed it *CUP-SHAPED COTYLEDON3* (*CUC3*). To understand the genetic interaction among *CUC1*, *CUC2* and *CUC3*, we generated the double and triple mutants. Mutation of *CUC3* enhanced the phenotypes not only *cuc2* but also *cuc1*, indicating that all three genes function redundantly during embryogenesis. The spatial and temporal expression patterns of *CUC3* almost overlapped with those of *CUC1* and *CUC2*. Interestingly, *cuc3* single mutant had abnormal shoots whose lateral organs distributed irregularly. An axillary shoot of *cuc3* occasionally separated from a base of cauline leaf and the inflorescence shoots sometimes failed to develop any axillary meristems. Both *cuc1* and *cuc2* mutations enhanced these phenotypes. These results suggest that *CUC3* is involved in axillary meristem ontogeny and in distribution of lateral organs together with *CUC1* and *CUC2*.

220 The *embryo yellow* mutant of *Arabidopsis thaliana* exhibits a defect in the cell shape and forms many adventitious leaves in the shoot apex

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We have isolated the *eye* (*embryo yellow*) mutant that has defects in the cell shape and the organization of the shoot apical meristem. While *eye* embryos appeared to develop normally up to the mature-embryo stage, they exhibited defects in the size and color in the maturation stage of the embryogenesis. Cells of *eye* mature embryos showed the irregular size and shape. The germinated *eye* plants were dwarf, and epidermal cells of the cotyledon and hypocotyl swelled. In the shoot apex of the *eye* mutant, a shoot apical meristem that consists of a dome of cells was not observed, and many adventitious leaves were formed. The *EYE* gene was predicted to encode a protein homologous to Cog7. In the mammalian, this protein is a subunit of a Golgi-localized COG complex that is required for normal Golgi morphology and function. This result suggests that phenotypes of the *eye* mutant might be caused by defects in the function of the Golgi apparatus, such as the biosynthesis of cell wall polysaccharides and the protein glycosylation.

221 SPLAYED is required for maintenance of the shoot apical meristem

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SPLAYED (SYD), a putative Snf2/Brm chromatin remodeling ATPase, was previously identified in a screen for enhancers of a weak *lfy* allele in *Arabidopsis thaliana* (Wagner D. and Meyerowitz E.M., *Curr. Biol.* 2002, 12, 85-94). Besides regulating floral homeotic gene expression as a LFY co-activator, SYD has additional roles in *Arabidopsis* development including maintenance of the shoot apical meristem (SAM), regulation of the onset of the reproductive phase, and control of ovule development. In this study, we have focused on investigating the role of SYD in maintenance of the SAM. When we compared SAM development in the *syd-2* null mutant to that in the L*er* wild-type, a reduction in SAM size was first observed in *syd-2* shortly after the onset of reproductive development. Consistent with the morphological defect in *syd-2* mutants, we observed significant reduction of mRNA levels in genes pivotal for SAM function, including *WUSCHEL*, *CLAVATA3* and *SHOOT MERISTEMLESS*. We therefore analyzed the phenotype of double mutants between *syd-2* and these SAM regulator mutants with respect to SAM organization, leaf initiation, and cotyledon fusion. Our data suggest that SYD controls maintenance of the SAM by regulating the expression of *WUSCHEL* and/or *SHOOT MERISTEMLESS*.

222 Mosaic analysis of cell proliferation mutations in flowers and leaves

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We are using a Cre-lox mosaic analysis system for observing the effects of cell division and cell growth mutations in floral meristems and leaves. Because they tend to cause embryo or seedling lethality, these mutations have not been studied in the adult plant. We are using lox-flanked wild-type genomic transgenes to complement specific mutants and bypass the lethality. Two different Cre recombinase lines have been developed to delete the complementary transgenes and create mutant clones late in development. To create floral specific sectors we use an AP3::Cre line that only expresses Cre recombinase in the B domain cells that give rise to the petal and stamen primordia. We are also using a conditional (heat shock) Cre recombinase to generate random sectors in vegetative tissue. Concurrently, we are characterizing an internal ribosomal entry site (IRES) to allow the ectopic expression of two genes from the same transcript. We are using two reporter genes expressed from a bicistronic message to determine if their activities are proportional to the level of message. We propose to use this technique to monitor the position and level of ectopic expression in mosaic sectors

223 The role of two novel MYB-domain genes in patterning lateral organs

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The Arabidopsis genes *AtMYB105* and *AtMYB117* encode R2/R3-type MYB domain proteins. The two proteins belong to the same sub-group of Arabidopsis MYB proteins, and additionally share a conserved domain outside of the MYB repeats. Examination of *GUS* reporter gene expression in enhancer trap lines containing insertions upstream of each *MYB* gene has given us information about the expression patterns of these genes. The expression of both genes is restricted to a domain between the shoot apical meristem and initiating leaf primordia during vegetative development and at the base of floral organs during reproductive development. This expression pattern may indicate that *AtMYB105* and *AtMYB117* function to establish a boundary between the meristem and initiating organs, or to regulate some aspect of communication between meristem and lateral organs. *AtMYB105* was also expressed in the anther sac, pollen, and ovary wall. Ubiquitous expression of *AtMYB105* under the control of the cauliflower mosaic virus *35S* promoter resulted in several abnormalities. *35S:AtMYB105* transgenic plants produced leaves that were rumpled, curled upward at the edges, and occasionally formed basal lobes. Upon flowering, the majority of *35S:AtMYB105* plants exhibited dramatic floral organ defects. Sepals and petals were reduced in size, such that the developing carpel was exposed. The carpels showed incomplete fusion, and produced ectopic ovules on the outer surface. This phenotype resembles that of mutations in the *CRABS CLAW* and *SPATULA* genes, and may indicate that ectopic expression of *AtMYB105* results in alterations in lateral organ polarity.

224 GCC7 a Nuclear Regulator of Glucosinolate Accumulation in Arabidopsis

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Glucosinolates are a class of sulfur-containing secondary metabolites found mainly in the Brassicales. Upon tissue damage, glucosinolates are hydrolyzed to biologically active products, including isothiocyanates, thiocyanates, epithiocyanates, and nitriles, which are proposed to act as allelochemicals and to play a role in plant defense. The cancer-preventive properties of natural isothiocyanates have renewed interest in glucosinolate biosynthesis. Using a bioassay we screened for mutants with altered glucosinolate content and composition and cloned the *GCC7* gene. Gain and loss-of-function mutations in the *GCC7* gene correlated with higher and lower levels of glucosinolate content, respectively. The predicted GCC7 protein comprises 454 amino acids and shares 30% sequence identity with the sunflower protein SF16. The GCC7 protein is part of a multi gene family whose members share at least one IQ motif, shown in mammalian systems to bind to calmodulin or other EF-hand proteins and to mediate regulation of a wide range of cellular processes by Ca2+-signals. The majority of these proteins contain a putative nuclear localization signal suggesting that they may function as regulators in the cell nucleus. Indeed transient expression of a GCC7::GFP fusion protein showed it is localized to the nucleus. The analysis of steady-state mRNA levels of several genes involved in glucosinolate biosynthesis showed that GCC7 protein modulates expression of several glucosinolate pathway genes. Histochemical analysis of GUS enzyme activity in a *GCC7* gene trap line revealed reporter gene expression exclusively in the vascular bundles of hypocotyls, leaves, stems and mature siliques. Interestingly, the observed tissue-specific pattern of *GCC7* promoter activity is strikingly similar to the expression patterns reported for several genes that encode enzymes involved in glucosinolate synthesis. Our data on gain and loss-of-function mutations of GCC7 as well as on its tissue-specific expression patterns and nuclear localization of the protein strongly suggest that *GCC7* plays a role in regulating glucosinolate accumulation.

225 LOB-DOMAIN genes regulate leaf development

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The formation of lateral organs from the shoot apical meristem (SAM) requires the activation of organ-specific genes and the suppression of meristem-specific genes. *KNOX* genes constitute one class of SAM-specific genes that are down-regulated during lateral organ initiation. Mutations in the Arabidopsis genes *ASYMMETRIC LEAVES1* (*AS1*) and *ASYMMETRIC LEAVES2* (*AS2*) result in a failure to maintain *KNOX*-gene repression in leaves. *as1* and *as2* mutants form rumpled, lobed leaves that curl downward and display vascular pattern defects. *AS2* encodes a member of the *LATERAL ORGAN BOUNDARIES* (*LOB*) gene family, which is defined by the conserved LOB domain. *LOB*, the founding member of this gene family is expressed between the SAM and initiating lateral organs, and is positively regulated by both *KNOX* genes and leaf-specific genes, including *AS1* and *AS2*. The *LOB* expression pattern suggests that it may function to regulate communication between the SAM and lateral organ primordia. We have found that ectopic expression of *AS2* results in both the transcriptional repression of *KNOX* genes and the adaxialization of lateral organs. Our results implicate *AS2* in the regulation of lateral organ polarity, something that was not uncovered by loss-of-function mutations. This suggests that *AS2* may act redundantly with respect to its role in polarity. Closely related *LOB-DOMAIN* family members may have overlapping functions with *AS2*. To address this possibility, we have initiated functional analyses of those *LBD* genes most closely related to *AS2*. Over-expression of *LBD36*, a closely related member of the *LBD* gene family, also results in *KNOX* gene repression. Our observations suggest a common role for a sub-class of *LBD* genes in *KNOX* gene regulation and leaf development.

226 The role of FUS3 in vegetative development of Arabidopsis thaliana

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The function of the *FUS3* gene as an important regulator of late embryo development and gene expression in Arabidopsis has been well-characterized (Keith et al., 1994; Meinke et al., 1994, Nambara et al., 2000). The loss-of-function mutation, *fus3*, leads to reduced storage protein levels and the failure to establish dormancy and desiccation tolerance in the embryo (Keith et al., 1994). The appearance of trichomes on cotyledons of *fus3* mutants suggests that there is an overlap between the late embryogenesis and vegetative developmental stages (Keith et al., 1994). The phenotypes of *fus3* has generated two interpretations regarding the role of *FUS3* during late embryogenesis. One involves a homeotic conversion event in which the cotyledon has been partially converted into a vegetative leaf (West and Harada, 1993). The other is a heterochronic interpretation which suggests that there has been an alteration in the timing of developmental events (Keith et al., 1994). A more comprehensive examination of the *fus3* mutant has revealed a defect in vegetative phase transition. In addition, *FUS3* expression has been observed in vegetative tissues. The vegetative phenotypes of *fus3* and *FUS3* expression outside of the embryo imply that *FUS3* performs a role during vegetative development and provide support for the heterochronic model. In order to isolate genetic interactors of *FUS3*, a suppressor screen of the *fus3* mutant has been performed. The screen has uncovered several classes of mutant phenotypes and their phenotypic characterization will be described.

227 *DAWDLE*, A New FHA Domain Gene in *Arabidopsis*

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The fork-head associated domain (FHA) is a phosphopeptide-binding domain found in a wide variety of organisms. Most characterized FHA domains, such as the ones found in yeast Rad53, are nuclear localized. The FHA domain interacts with the transcription machinery, functioning in cell cycle and DNA damage response pathways. The only characterized FHA domain in Arabidopsis is found in Kinase Associated Protein Phosphatase (KAPP), a phosphatase involved in signal transduction pathways which affect plant growth and development. KAPP uses the FHA domain to interact with receptor-like kinases (RLKs) at the cell membrane. To better understand the developmental function of FHA domains in Arabidopsis, we are taking a functional genomics approach. Including KAPP, there are 14 predicted FHA domain signature (FDS) genes in the Arabidopsis genome. We searched several T-DNA populations for insertions in the 13 additional FDS genes and have examined phenotypes of plants that contain loss of function alleles. Plants homozygous for the T-DNA insertion in the gene *dawdle* (At3g20550) are developmentally delayed, have curly leaves, abnormal flowers and decreased fertility. The DAWDLE cDNA predicts a 35kD protein that contains only a nuclear localization signal and an FHA domain. Preliminary data suggests that DAWDLE (DDL) may be the first Arabidopsis FHA domain containing protein shown to function in the nucleus

228 Over-expression of a novel small peptide ROTUNDIFOLIA4 decreases cell proliferation and alters leaf shape.

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The formation of plant leaves is largely determined by polar cell expansion along the leaf-length and leaf-width directions, which are under independent genetic control. However, the roles of polar cell proliferation in leaf morphogenesis are largely unknown. A dominant *Arabidopsis*mutant, *rotundifolia4-1D* (*rot4-1D*) was isolated from a gain of function mutagenesis experiment. *rot4-1D* mutants possessed short leaves and floral organs and had fewer cells specifically in the leaf-length direction. *ROT4* encodes a novel small peptide whose ORF had not been predicted in the Arabidopsis genome annotation. Several predicted genes that are similar to ROT4 were found in Arabidopsis genome and were named *ROT FOUR LIKE1* (*RTFL1*) through 22. Interestingly, *RTFL* were found only in seed plants genome and ESTs. All *RTFL*s contain a conserved region that were named RTF domain. The RTF domain of ROT4 is at the position from the 17th to the 45th amino acid residue. Peptides that become from the N terminus to the 45th amino acid residue of ROT4 and from 17th amino acid residue to the C terminus of ROT4 were named ROT4δC and ROT4δN, respectively. The transgenic plants that express *ROT4δC* or *ROT4δN* under control of the Cauliflower Mosaic Virus 35S promoter show rot4 phenotypes. A ROT4-GFP fusion protein was localized in plasma membrane in onion epidermal cells. These results suggest that leaf length of seed plants is controlled not only by polar cell expansion but also by polar cell proliferation. Thus, ROT4 indicates an important role for polarity-dependent cell proliferation in leaf morphogenesis.

Reference: Narita et al. (submitted).

229 Evolution of the *REVOLUTA*-like family of Homeodomain Leucine-Zipper Genes

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Class III homeodomain leucine-zipper (HD-ZIP III) genes are involved in meristem initiation, organ polarity establishment, and vascular development. To complement genetic analyses being carried in Arabidopsis, we have identified additional members of this gene family in diverse plants including a moss (*Physcomitrella patens*), a lycophyte (*Selaginella krausiana*), and a gymnosperm (loblolly pine). Based on phylogenetic analysis using these new sequences and five genes in both of the sequenced genomes of Arabidopsis and rice, the relative timing of gene duplication events can be determined. For example, the first persisting HD-ZIP III gene duplication event occurred in an ancestral vascular plant prior to the divergence of gymnosperms and angiosperms. This event gave rise to the REV/PHB/PHV and ATHB8/ATHB15 clades. By comparing the expression patterns and, eventually, the functions of these genes in non-flowering plants to that of the Arabidopsis paralogs; we will gain a better understanding the roles of gene duplication and functional divergence in the evolution of HD-ZIP III genes and also provide a specific example of how these forces may shape key developmental regulators during the course of plant evolution.

230 Intra-vital imaging reveals spatial and temporal dynamics of cell behavior in the shoot apical meristems of Arabidopsis thaliana

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The shoot apical meristem (SAM) acts as a continuous source of cells for all the above ground plant parts(1). According to the anatomical and cytological studies, SAM can be broadly sub-divided into central zone (CZ), peripheral zone (PZ) and rib-meristem. The CZ has been postulated to harbor stem-cells, while cells in the PZ are committed to form organ primordia. The rib-meristem cells are incorporated into the developing stem. The presence of clonally distinct layers of cells adds an additional level of organization to the SAM. Thus SAM is a dynamic structure with cells constantly changing their position and acquiring distinct fates. Remarkable feature of SAM is that the relative sizes of each of the domains are maintained, across clonally distinct layers, amidst the flux of cells. How is this co-ordination achieved both within and across layers is still a mystery. It is believed that the co-ordinated cell division, cell expansion and cell displacement patterns play a crucial role in maintaining the size of SAM. However, the studies on SAMs so far are static and two-dimensional in nature. The dynamic nature is either lacking or less understood(2). Analyzing the spatial and temporal dynamics of cell behavior in living plants should yield new insights into the underlying mechanisms. This study is aimed at analyzing cell behavior in SAMs of developing wild type plants. We have devised a method to visualize cells in the developing SAMs. We have followed “every cell” in SAM for several days. We will present an integrated spatial-temporal map of cell behavior. We will discuss the results and ongoing efforts in integrating cell behavior to gain insights into meristem maintenance.

1. Meyerowitz, E.M. (1997) Genetic control of cell division patterns in developing plants. Cell, 88(3): 299-308
2. Soma, K. and Ball, E. (1963) Studies of the surface growth of the shoot apex of Lupinus albus. Brookhaven Symp Biol. 16, 13-45.

231 Identification of *cis*-elements in the *AtSUC2* promoter

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In *Arabidopsis* leaves, the *AtSUC2* promoter drives the cell-specific expression of the *AtSUC2* sucrose-H+ symporter in companion cells of the phloem. This expression is strongly dependant on the developmental state of the leaf during sink/source transition, starting in the tips of young leaves and proceeding to the bases in mature leaves. Thus, *AtSUC2* expression is restricted to source leaves or the source part of transition leaves. We have chosen this expression of *AtSUC2* as a model system to study the regulation of sink/source transition, which marks an important metabolic switch during leaf development. The objective of this study is the identification of *cis*-acting elements in the *AtSUC2* promoter responsible for the described expression pattern. Using the *GFP* reporter-gene as marker in deletion analyses of the *AtSUC2* promoter, we identified a 70-bp region containing sequence motifs essential for *AtSUC2* expression. We then used a minimal-promoter-system and the *GUS* reporter-gene to examine this 70-bp promoter region and its adjacent sequences in more detail in linker-scanning analyses. Two regions (14 bp and 16 bp, respectively) turned out to be essential, because mutating only one of them already was sufficient to completely abolish expression of the *GUS* reporter. Database search revealed homology of these two sequences to known transcription factor binding-sites. At present these two motifs are modified in the original promoter using site directed mutagenesis in order to confirm the results obtained with the minimal-promoter-system. Furthermore both sequences are used as probes in the screening of a cDNA expression library for binding of potential transcription factors.

232 *POLTERGEIST* (*POL*) and *POLTERGEIST-LIKE1* (*PLL1*) are essential for the establishment of both shoot and root meristems in Arabidopsis

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All above-ground organs of Arabidopsis are generated from shoot apical meristems whose size is regulated by the CLAVATA1 (CLV1)-WUSCHEL (WUS) signaling pathway. *pol* mutations were isolated as suppressors of stem cell accumulation in *clv* mutants. *POL* encodes a novel type of protein phosphatase 2C. *pol* single mutants have almost no detectable phenotype. To determine if *POL* function was redundantly performed by any of the five *POLTERGEIST-LIKE* (*PLL*) genes in Arabidopsis, mutants for each of the *PLL* genes were isolated. *pll1* mutants exhibited weak suppression of Clv^- phenotypes when crossed to *clv1*, *clv2*, and *clv3*. The transgenic expression of the *PLL1* gene under the control of the *POL* promoter complemented the Pol^- phenotype in a *clv2-1 pol-6* background. While *pll1* single mutants have no phenotype, *pol pll1* double mutants are seedling-lethal, having neither shoot meristem nor root meristem. The earliest defects detected in *pol-6 pll1* embryos occurred in the mid-globular stage when the hypophysis and procambial cells of the *pol-6 pll1* embryos divide rather symmetrically while those of wild-type embryos divide asymmetrically. The shoot meristem of *pol-6 pll1* exhibited a *wus*-like stop-and-go phyllotaxis when it was grafted onto roots of a wild-type plant. These results suggest that *POL* and *PLL1* are functionally redundant and play essential roles in the formation and maintenance of plant meristems.

233 The boundary between the shoot apical meristem and lateral organs

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The normal development of leaves and other lateral organs requires the simultaneous repression of meristem-specific genes and the activation of organ-specific genes. This process must therefore involve communication between the shoot apical meristem and initiating lateral organ primordia. We have identified several Arabidopsis genes that are expressed in a domain between the meristem and initiating lateral organs, in a pattern suggestive of a role in communication between the two groups of cells. One of these genes, *LATERAL ORGAN BOUNDARIES* (*LOB*) encodes a novel, plant-specific protein. The LOB protein contains a conserved ~100 amino acid domain (the LOB domain) that is present in 42 additional Arabidopsis proteins. While the biological role of LOB remains unknown, it is clear that the closely related *ASYMMETRIC LEAVES2* (*AS2*) gene is important for both the development of normal leaf shape and for the repression of *KNOX* genes in the leaf. To date, *as2* is the only *LOB*-domain gene that shows a clear loss-of-function phenotype, indicating that there is likely to be functional redundancy among genes in this family. *LOB* expression is positively regulated by the *KNOX* genes *BREVIPEDICELLUS* (*BP*) and *SHOOT MERISTEMLESS* (*STM*), as well as by *ASYMMETRIC LEAVES1* (*AS1*) and *AS2*. The LOB protein is nuclear-localized, and we have shown that it interacts with AS1 and two other MYB-domain proteins, MYB105 and MYB117. Both *MYB105* and *MYB117* are expressed at the base of lateral organs in a "*LOB*-like" pattern, and are negatively regulated by *BP*. The genetic interactions between *LOB*, *KNOX* genes, *MYB105* and *MYB117*, *AS1* and *AS2* highlight the complexity of the regulatory networks controlling communication between the shoot apical meristem and lateral organ primordia. Ectopic expression of *AS2* results in the repression of *KNOX* genes, and in the formation of adaxialized lateral organs. Ectopic expression of *MYB105* results in floral organ defects such as incomplete carpel fusion and the production of ectopic ovules, phenotypes that also suggest defects in adaxial-abaxial polarity. Together these data provide evidence that genes expressed between the meristem and lateral organs play a role in the regulation of adaxial-abaxial polarity.

234 Modes of intercellular transcription factor movement in the Arabidopsis apex

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One of the most recent and intriguing discoveries in plant biology has been the movement of transcription factors between cells. In *Arabidopsis thaliana*, the floral identity protein LEAFY has strong non-autonomous effects when expressed in the epidermis, mediated by its movement into underlying tissue layers. In contrast, a structurally unrelated floral identity protein, APETALA1, has only limited non-autonomous effects. Using GFP fusions to monitor protein movement in the shoot apical meristem and in floral primordia of *Arabidopsis*, we found a strong correlation between cytoplasmic localization of proteins and their ability to move to adjacent cells. The graded distribution of several GFP fusions with their highest levels in the cells where they are produced is compatible with the notion that this movement is not regulated by a specific mechanism. We also present evidence that protein movement is more restricted laterally within layers than from L1 into underlying layers of the *Arabidopsis* apex. Based on these observations, we propose that intercellular movement of transcription factors can occur in a non-targeted fashion due to simple diffusion. This hypothesis raises the possibility that diffusion is the default state for many macromolecules in the *Arabidopsis* apex, unless they are specifically retained.

235 Ectopic Expression of the Ubiquitin C-terminal Hydrolase UCH1 Alters Shoot Architecture in *Arabidopsis thaliana*

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The ubiquitin/26S proteasome pathway is an essential pathway for many aspects of eukaryotic cell growth and development by degrading important cell regulators conjugated by multiple ubiquitins(Ub). Deubiquitinating enzymes are critical for this pathway by controlling the steady-state levels of Ub and Ub-protein conjugates. The ubiquitin C-terminal hydrolases (UCHs) are one subfamily defined by the presence of a signature active site bearing a Cys/His/Asp catalytic triad. DNA database searches with the UCH concensus sequence revealed that the Arabidopsis thaliana genome contains two UCH genes, designated *UCH1* and *2*. The encoded ~330 amino acid proteins are 74% similar to each other. Their closest orthologs are members of the UCH37 family , a group of hydrolases that potentially associate with the 26S proteasome and helps shorten poly-ubiquitin chains before target degradation. Like the UCH37 family, UCH1 and 2 contain two UBA domains and a conserved C-terminal extension appended to the catalytic core. This extension has been proposed to facilitate interaction of UCH37 with the 26S proteasome by binding the RPN12 Lid subunit. Arabidopsis UCH2 has hydrolase activity *in vivo* and *in vitro* and is capable of cleaving both α-peptide and ε-peptide bonds involving the C-terminus of ubiquitin. Interestingly, plants ectopically-expressing UCH1 develop a bushy phenotype caused by an increase in axillary growth and reduced shoot internode elongation. The phenotype suggests that UCH1/2 may help regulate cell division and expansion in Arabidopsis by trimming poly-ubiquitin chains from regulatory proteins thus inhibiting their degradation by the 26S proteasome. However, the UCH1/2 proteins are not part of the Arabidopsis 26S proteasome purified by conventional methods suggesting that they associate loosely if at all with the complex *in vitro*. Experiments are in progress to understand the molecular mechanism responsible for the UCH1-ectopic expression phenotype. In addition, we have also identified T-DNA mutants disrupting the *UCH1* and *2* genes. No obvious phenotypes have been observed in single homozygous mutants, suggesting that *UCH1* and *2* have redundant functions. The isolation and characterization of double homozygous *uch1uch2* mutants are underway.

236 A novel protein localizing in the envelope membrane of plastid is involved in both of the plant morphogenesis and plastid division

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The *crumpled leaf* (*crl*) of *Arabidopsis thaliana* was isolated as a mutant showing dwarfism, pale green color, and wavy leaf shape. Our histological analysis revealed that planes of cell division were distorted in shoot apical meristem, root tip, and embryo in the *crl* mutant. Differentiations of cortex and endodermis cells in inflorescence and endodermis cells in root were also disturbed in the *crl* mutant. These results suggested that the *CRL* gene concerns with the plant morphogenesis through cell differentiation and determination of cell division plane. In addition, all the plastids we examined were enormously enlarged and reduce its number whereas the biogenesis of chloroplasts were morphologically normal in the *crl*. The *CRL* gene encoded a novel protein of molecular mass of 30 kDa. To examined the subcellular localizations of the CRL protein , we fused the *GFP* to the 3' end of the entire coding region of the *CRL* and introduced it into Arabidopsis. The fusion protein (CRL-GFP) was localized at the chloroplast periphery suggesting that the CRL protein localized in the envelope membrane of plastids. The same pattern of localization was observed when the *GFP* was fused to the 5' end of the *CRL* suggesting that the CRL had no cleavable signal sequence at its ends. When we fractionated isolated chloroplasts into the soluble and membrane fractions and detected the CRL-GFP protein by immunoblot, the CRL-GFP protein was present in the membrane fraction. The CRL-GFP protein was digested by the trypsine treatment of isolated chloroplasts. These results confirmed the localization of the CRL protein in the envelope membrane of chloroplast. A possible correlation between plant development and plastid division will be discussed.

237 LSH1, the Member of a New Gene Family Involved in Phytochrome Signal Transduction

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As an important environmental factor, light regulates plant growth and development through a network of endogenous factors. From screening Arabidopsis activation tagged lines we isolated a dominant mutant (*lsh1-D*) that showed hypersensitive responses to continuous red (cR), far-red (cFR) and blue (cB) light and cloned the corresponding gene, *LSH1*. *LSH1* encodes a nuclear protein of a novel gene family that has homologs in *Arabidopsis* and rice. The effects of *lsh1-D* mutation were tested in a series of photoreceptor mutant background. The hypersensitivity to cFR and cB conferred by *lsh1-D* was completely abolished in a mutant *phyA* null background (*phyA-201*) and the hypersensitivity to cR and cFR conferred by *lsh1-D* was much reduced in a phytochrome-chromorphore synthetic mutant *hy1-1* background. These results indicate that LSH1 mediates FR- and B-light-responses dependent on phyA and that LSH1 also requires functional phytochromes for mediating R-light responses. Our data provide evidence that LSH1 is a novel component positively involved in phytochrome-mediated light signaling. And we will provide data suggesting that LSH1 might be also involved in shoot meristem identity.

238 HANABA TARANU,a GATA transcription factor that regulates flower development

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GATA transcription factors are known to play important and diverse roles in animal and fungi. However, their function in plants is scarcely known. We isolated a new mutant, hanaba taranu (han), which affects both flower development and shoot apical meristem (SAM) development in Arabidopsis thaliana. Mutants have fused sepals, reduced organ numbers in the 2nd (petal) and 3rd (stamen) whorls, as well as carpel fusion defect. In addition, the han mutation interacts strongly with the clavata (clv) mutations (clv1, clv2 and clv3), resulting in more fascinated SAM and more severe floral defects. Cloning the HAN gene revealed that it encodes a GATA-3 like transcription factor with a single zinc finger domain. Consistent with its role in promoting organ separation and organ identities, HAN is transcribed in the boundaries between the meristem and its newly initiated organ primordia, and also in the boundaries between different floral whorls. It is also expressed in the vascular tissues, developing ovules and the embryo. Ectopic expression of HAN causes growth retardation and loss of meristem activity, further supporting its roles in restricting stem cell activity and in regulating cell growth/differentiation during normal development.

239 A *lonesome highway* leads to root polarity

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The Arabidopsis root has a stereotyped radial arrangement of tissues. From the outside in, these are epidermis, cortex, endodermis, pericycle and the vascular tissues xylem and phloem. Superimposed on the radial symmetry of the root is an axis defined by the linear arrangement of xylem elements. In Arabidopsis, the root has two xylem poles (diarch), and the lateral roots emerge from pericycle cells adjacent to the xylem poles. In a screen for mutations that altered the expression of a GFP marker expressed in the files of pericycle cells that make lateral roots , we identified two loci, *lonesome highway (lhw)* and *passing lane*. *lhw* mutants express the GFP marker in only one file of pericycle cells, produce lateral roots from only one side of the primary root, and have only one strand of protoxylem (ie. they have one instead of two xylem poles). *lhw* plants are healthy and fertile and exhibit only a slightly shortened and agravitropic adult stature. *lhw* roots exhibit a normal auxin response in primary root shortening and production of lateral roots, however exogenous IAA or 2,4D does not rescue the xylem or pericycle phenotype. *LHW* is a member of a plant specific gene family whose members encode proteins with no obvious functional motifs. However, expression of several of the family members, including *LHW*, appears to be regulated in response to treatments that promote xylem or lateral root formation.

Mutations in several other genes also disrupt aspects of radial pattern. The SHR and SCR transcription factors are required to establish cell fate in the endodermis and cortex[1,2], and the cytokinin receptor WOL /CRE1 is required to promote divisions that lead to proper phloem and xylem pattern[3]. *SCR* was recently shown to have a role in QC identity and *scr* primary roots are determinate[4]. We have found that *lhw* roots are also determinate and that a *SCR::GFP* transcriptional reporter is missing from the QC, although other QC markers are present. *LHW* expression and the relationship between *LHW* and the radial patterning genes *SHR, SCR* and *WOL* will be presented.

1 Helariutta et al. (2000) CELL 101: 555-567;
2 DiLaurenzio et al (1996) CELL 86: 423-433;
3 Mahonen et al. (2000) G& D 14: 2938-2943;
4 Sabatini et al. (2003) G& D 17: 354-358

240 A vast source of genetic redundancy: Genome-wide cell-type specific express

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Gene expression provides insight into gene function and can be used comparatively to examine genome evolution. For example, duplicated gene pairs that continue to be transcribed may retain the same expression pattern (and possibly the same function), or they may evolve different expression patterns, indicating a divergence in their roles. Despite longstanding interest in the role of gene duplication in evolution, little is known about the comparative expression of gene pairs after duplication on a large scale in multi-cellular eukaryotes. We first present a new technique to determine genome-wide cell and tissue-type expression, which we used to examine the expression patterns of 1,000 gene pairs of different ages in the *Arabidopsis* root. We found a rapid divergence in the expression patterns of about 25% of gene pairs, but the vast majority of duplicates retained a stable positive correlation over tens of millions of years, suggesting that selection either maintains co-expression or constrains expression divergence, or both. Gene function appeared to influence which type of expression pattern between duplicates emerged.

241 BUDDY controls plant cell polarity and growth directionality

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Root hairs elongate by tip growth, a highly polarised form of growth. Mutants with defects in hair morphology could be useful for understanding how cell polarity is established and maintained. The *buddy* (*bdy*) mutants of Arabidopsis do not produce long polarised root hairs, but instead form rounded structures with multiple points of growth, or short curved hairs. So BUDDY is a gene product which is required for the establishment and maintenance of tip growth. The Bdy^- phenotype is additive with those of the epidermal patterning mutants, *ttg*, *gl2*, *wer* and *cpc*, suggesting that BDY acts downstream of these regulatory genes. Treatment of roots with auxin and ethylene can rescue *bdy* hair elongation, but not growth directionality. Three *bdy* alleles have similar phenotypes. *bdy1-1* and *bdy1-3* are presumed nulls, but *bdy1-2* has a mutation which causes an amino acid substitution in a conserved residue. The mapping, cloning and characterisation of *BUDDY* and other root hair genes will be presented and their potential roles in tip growth and cell polarity discussed.

242 Expression Analysis in roots of *Arabidopsis thaliana* by Gene Trap Mutagenesis

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In order to establish a system for characterizing gene function by utilizing the genome sequence information, we have generated a large number of T-DNA insertion lines using a newly constructed binary vector according to the vacuum infiltration transformation method. The vector carries a *uidA* [β-glucuronidase (GUS)] reporter gene which allows monitoring of promoter activity of the inserted genes, a transposable element *Ds* for generation of knock-out, and the *cis* sequences required for *Agrobacterium*-mediated transformation. We have generated a total of 57,000 transgenic lines. Approximately 8% of the 20,000 plants tested for GUS activity exhibited positive staining. We have been analyzing the insertion lines which exhibited GUS staining in roots. The flanking region of the GUS gene was amplified by inverse PCR or adapter mediated PCR, and subjected to sequence analysis. To date, we have identified 20 genes which promote GUS expression in roots. We examined the root growth of these lines. Reduced root growth rate compared to the wild type was observed for a line showing GUS activity in the root tip. In this line, the T-DNA was inserted in an ABC protein gene. To assess the root growth in other knockout lines of the ABC protein gene, we have screened and isolated 3 additional lines. Reduced root growth rate was also observed in these lines. These results indicate that the ABC protein gene plays an important role in root growth and development.

243 Pattern and timing of expression of a root tissue marker during *in vitro* shoot development

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In vitro organogenesis is a valuable means of propagating plants asexually and provides a useful system for studying the development of higher plants. However, very little is understood about the process that drives *de novo* organ formation in cultured tissue. In an attempt to elucidate the developmental changes that occur during *in vitro* shoot organogenesis, root explants from a transgenic Arabidopsis thaliana seed line with a root GUS reporter gene construct were induced to form shoots in a two-step tissue culture system. This seed line exhibited strong GUS expression in the elongation zone of seedling and adventitious root tips, and was considered a marker for root identity. The cultured tissue with this GUS marker for root identity showed expression in root tissue, callus cells, and the edges of expanding leaves produced during *in vitro* shoot organogenesis. However, the root tissue marker did not show GUS expression in the SAM of adventitious shoots. Thus our root marker appears to mark areas of non-meristematic cell division and cell enlargement and elongation, so perhaps the true function of this root marker gene is closely related to these cellular functions.

244 *ROT120* encodes a putative GPI-anchored protein involved in stimulus-induced root hair elongation.

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Root hairs are important not only to aid the uptake of nutrient and water but to anchor roots in soil. When *Arabidopsis* roots come away from the surface of agar medium (off surface), newly formed root hairs become longer and tilted toward the root tip. This stimulus-induced developmental change can be considered for roots to keep contact with soil. To investigate the mechanisms by which the architecture of root hairs changes in response to environmental conditions, we have isolated mutants defective in the formation of off-surface-root hairs. A novel recessive mutant *rot120* produces off-surface-root hairs dramatically shorter than on-surface ones, that is opposite to that wild type produces longer off-surface hairs than on-surface ones. Map-based cloning revealed that the *ROT120* gene encodes a putative GPI-anchored protein of unknown function.

A possible role of the ROT120 protein in the formation of off-surface-root hair will be discussed.

245 Expression profiles of vascular development-related genes in *Arabidopsis* root

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Vascular system in plants consists of various types of cells, such as tracheary elements (TEs), sieve elements, companion cells, xylem and phloem parenchyma cells, and fibers. We have found more than 200 genes expressed specifically during vascular development using cDNA microarrays of the *Zinnia in vitro* culture system in which xylem differentiation is induced from isolated mesophyll cells. In this study, we searched *Arabidopsis* genes with the vascular expression. We selected 123 *Arabidopsis* genes with significant sequence similarity to the *Zinnia* genes, expression profiles of which were analyzed in transgenic *Arabidopsis* plants carrying reporter genes (*YFP*, *CFP*, *GFP*, and *GUS*) driven by the promoters of the *Arabidopsis* genes. Because *Arabidopsis* root has a simple structure and transparent tissues and is a good model for analyzing development of vascular system, we mainly observed the expression profiles in roots. Among the 123 genes analyzed, we found 87 genes with the expression in vascular cells, among which a lot of genes were expressed specifically in xylem cells at different developmental stages, including early and late procambium, early protoxylem poles, and differentiating TEs. These genes will be invaluable markers of vascular development, and also be new clues to elucidate the molecular mechanisms underlying vascular development. Based on the results, we will discuss the developmental stages of vascular system in *Arabidopsis* roots.

Demura *et al*. (2002) PNAS 99 15794-15799

246 Analysis of R3-type MYB genes for epidermal cell differentiation

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CAPRICE (*CPC*), encodes a small protein with R3 MYB motif and promotes root-hair cell differentiation in *Arabidopsis* (Wada et al. Science (1997) 277, 1113). We showed that the CPC protein moves from hairless cell to neighboring hair-forming cell and represses the expression of the homeodomain-leucine zipper gene, *GLABRA2* (Wada et al. Development (2002) 129, 5409). To understand the mechanism of cell-to-cell movement of CPC, we constructed several plasmids in which truncated CPC was fused to GFP, and examined which region of CPC is responsible for cell-to-cell movement. Two motifs, one in N-terminal region and the other in Myb domain, are required for cell-to-cell movement. The latter domain also functions as a nuclear localization signal. To investigate the direction of CPC movement, CPC:GFP fusion protein was expressed in the endodermis or hair-cell by *SCARECROW* or *AtMYC2* promoter, respectively. The results showed that GFP fluorescence was observed in all epidermal cells.

We found four *CPC*-like Myb sequences in Arabidopsis genome including *TRIPTYCHON*. Overexpressers of these four genes showed the reduction of the number of trichome and the increase of the number of root-hair, which is similar to *35S*::*CPC* plant (Wada et al. Science (1997) 277, 1113). Promoter::*GUS* analyses indicated that they were specifically expressed in epidermal cells. These results suggest that *CPC*-like R3 MYB genes cooperatively regulate epidermal cell differentiation in Arabidopsis.

In contrast with *CPC*, R2-R3-type MYB gene, *WEREWOLF* (*WER*) negatively regulates the root-hair formation. To understand the difference between activation and repression by MYB transcription factors in plants, we made the chimera constructs between CPC and WER. As a result, CPC R3 motif did not substitute for WER R3 motif, and WER R3 motif did not also substitute for CPC R3 motif. We will discuss the relationship between the ability of cell-to-cell movement of CPC and the activity of CPC for promoting root-hair cell differentiation.

247 Expression profiling of auxin-treated roots reveals a growth signature and suggests multiple mechanisms for IAA homeostasis

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Treating arabidopsis roots with exogenous auxin results in massive changes in cellular processes, including increased cell division and expansion at sites of *de novo* lateral root formation, and inhibition of elongation behind the root tip. A timecourse of microarray experiments on 14-day-old roots treated with 10 µM IAA revealed about 100 genes that are substantially and consistently upregulated over the first few hours following auxin treatment. Increased mRNA accumulation of a gene coding for an expansin together with pectin modifying enzymes suggests a mechanism for inducing substantial increases in cell wall extensibility; genes encoding proteins involved in lipid formation and vesicle delivery may speak to alterations in the processes associated with membrane formation; and increased expression of genes coding for ribosomal proteins and elongation factors support a general increase in metabolism. Increased mRNA accumulation for genes encoding transcription factors and possible components of signal transduction pathways were also observed. In addition to these potentially growth-associated genes are genes whose cognate proteins may be involved in regulating cellular levels of IAA. One way in which cells could restore IAA levels in the aftermath of exogenous application is to increase the rate of auxin transport. Such a hypothesis is supported by the increases in mRNA accumulation for several auxin transporters seen here. Another intriguing possibility is that high levels of IAA may stimulate IBA formation. One of the genes upregulated here, 3-ketoacyl CoA thiolase, encodes a protein that may be involved in interconversion of IAA and IBA. A second gene encodes a protein involved in valine catabolism, a separate pathway that has been also implicated in IBA metabolism. Further investigations into the functions of these auxin-regulated genes are underway.

248 Studying the Circumnutating Movements of Arabidopsis thaliana Roots on the Random Positioning Machine (two axes clinostat)

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The root growth habit of two *Arabidopsis* wild-types ecotypes (Wassilewskija and Columbia), of the left handed mutant *1-6C*, and of the auxinic and gravitropic mutants *aux1, eir1* and <I/rha1 was studied on the RPM, comparing it with 1g conditions. The primary root pattern of 7 day old roots grown on vertically set hard-agar plates was considered. By 1 g conditions, as it was already known, and confirmed through the present experiments, the primary roots of the wild-types grew by slanting to the right-hand, those of the mutant *1-6C* by slanting to the left-hand, and those of the mutants *aux1* and *eir1* through random movements. On the other hand, the roots of the *rha1* mutant grew down the dishes along an almost straight line. By contrast, on the RPM the wild-type ecotypes roots produced large loops to the right-hand, the mutants *1-6C* loops to the left-hand, and the auxinic mutants *eir1*, *aux1* and *rha1* just random movements. The primary roots of the wild-type plants and *1-6C* mutants thus appeared animated by a form of chiral circumnutation, which was lacking in the auxinic and gravitropic mutants. Consequently, it is suggested that the plant hormone auxin is involved not only in gravitropism, as is known, but also in root chiral circumnutation. It is also proposed to distinguish the process of chiral circumnutation of plant roots from that of random nutation. In addition, gravity appeared to regulate the chiral circumnutating movements, since the scatter of the circumnutating patterns among the different samples, was significantly lower in 1g conditions in the wild-type ecotypes than in the gravitropic and auxinic mutants.

249 WVD2 is a novel plant protein that affects anisotropic cell expansion and helical growth

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Arabidopsis roots exhibit characteristic growth patterns in response to gravity and asymmetric mechanical stimuli; for example, roots of most ecotypes skew to the right and form sinusoidal waves on tilted agar surfaces. We have identified the gain-of-function mutant *wave-dampened 2-1* (*wvd2-1*), which shows root wave dampening and leftward skewing (Yuen et al., Plant Physiol 2003 131:493). *wvd2-1* has impaired anisotropic cell expansion in multiple organs. Cortical microtubules are oriented aberrantly in regions of *wvd2-1* roots. WVD2 is a small, highly charged novel protein containing a conserved region predicted to form a coiled-coil structure. It is a member of a small family of predicted proteins in *Arabidopsis* and a larger family in dicots, monocots, and lower nonvascular plants. The PROPSEARCH algorithm, which detects remote homologs using protein compositional features, indicates compositional similarity between WVD2 and SCG10-like proteins, which in animals affect neuronal elongation by influencing microtubule stability. Biochemical studies indicate that purified epitope-tagged WVD2 protein co-sediments with microtubules during in vitro binding assays, and data will be presented on the affect of WVD2 on microtubule stability. Additionally, we are assessing the causes of altered root growth in *wvd2-1* by quantifying the spatial distribution of elongation and rotation and the orientation of cellulose microfibrils. Finally, we will discuss results of biochemical, genetic, and yeast two-hybrid approaches aimed at identification of proteins that interact with WVD2. We will discuss the role of WVD2 and cortical microtubules in the regulation of anisotropic cell expansion and spiral root growth in *Arabidopsis*.

250 A high affinity nitrate transporter regulates lateral root initiation

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The architecture of root systems is greatly influenced by environmental conditions. Therefore, we use the Arabidopsis root system to study developmental plasticity. Both the number and placement of lateral roots are highly responsive to nutritional cues; however, very little is known about how plants interpret complex environmental conditions and make the decision to form lateral roots. We found that when Arabidopsis seedlings are grown on nutrient media with a high sucrose to nitrate ratio lateral root initiation is dramatically repressed. A mutant, lateral root initiation 1 *(lin1)*, was isolated that produces more lateral roots under this repressive condition. Here we describe the cloning of the *LIN1* gene. PCR-based mapping placed the mutation near the top of Chromosome I. Fine mapping and sequence analysis revealed a missense mutation (G119R) in the *NRT2.1* gene (At1g08090). Phenotypic analysis of Salk T-DNA insert lines (Salk_008253 and Salk_035429) confirmed that the mutation in the *NRT2.1* gene was responsible for the phenotype seen in the *lin1* mutant. *NRT2.1* encodes a high-affinity nitrate transporter. It is highly expressed in the roots, and it has been shown that its expression level is induced by low external nitrate concentrations. This is consistent with the fact that the *lin1* phenotype is only seen under low nitrate conditions. However, a mutant defective in a high-affinity nitrate transporter should have less nitrate uptake, but the *lin1* mutant produces more lateral roots on the repressive media. The *lin1* mutant also produces more lateral roots on media with no added nitrate, showing that the *lin1* phenotype is independent of external nitrate. Together, these results suggest that NRT2.1 is not only a nitrate transporter, but is also involved in a signal transduction pathway that coordinates lateral root initiation with nutritional cues.

251 Regulated KRP1 degradation and early lateral root initiation in *Arabidopsis*

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In *Arabidopsis*, lateral roots originate from pericycle cells located at the xylem poles. During the early stage of lateral root formation, pericycle founder cells, which are located at the opposite xylem poles, are activated and undergo cell division to initiate lateral root formation. Although detailed morphological analysis data have been obtained about the lateral root formation in *Arabidopsis*, the cell cycle regulation associated with pericycle cell activation and early lateral root initiation is not well understood. In order to understand the molecular mechanism of pericycle cell activation during early lateral root initiation, the function of cyclin-dependent kinase inhibitor *KRP1* in early lateral root initiation was investigated. The *GUS* reporter gene expression driven by the *KRP1* promoter and *in situ* hybridization showed that *KRP1* is expressed ubiquitously in various tissues including roots, hypocotyls, cotyledons, leaves, flowers, and siliques. The reduced formation of lateral roots in *KRP1* overexpression transgenic plants suggested that *KRP1* may play an important role in lateral root formation. In addition, *KRP1* overexpression could inhibit auxin-promoted lateral root formation. An accumulation of KRP1-GUS fusion proteins in plants treated with the 26S proteasome inhibitor MG132 indicated that KRP1 is degraded by the 26S proteasome. Furthermore, the accumulation of KRP1-GUS fusion proteins in mutants deficient in ubiquitin-dependent protein degradation in plants including *axr1-3*, *CSN5* antisense line, *axr6-1*, and *ask1-1* suggested that KRP1 is degraded by a SCF ubiquitin protein-ligase which is regulated by the AXR1-dependent Rub conjugation pathway. Our results indicate that the ubiquitin/26S proteasome pathway may regulate KRP1 degradation to activate pericycle cell division and initiate lateral root formation during early lateral root formation.

252 *RHD6* is required for normal root hair development in *Arabidopsis*

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Root hairs in *Arabidopsis* are produced by specialised root epidermal cells. These cells are arranged in files alongside files of non hair cells. The fate of each epidermal cell is determined by differentially expressed transcription factors and reinforced by positional signaling. The screening and description of mutants with abnormal hair pattern or shape have enabled the identification of players in the genetic control of this model cell.

Here we desrcibe a gene defined by the *rhd6-2* mutation. Roots homozygous for *rhd6-2* have fewer and shorter root hairs than WT implying a role for *RHD6* in root hair development. In this study we describe the phenotypic analysis, the genetic analysis and the preliminary molecular analysis of three *rhd6* alleles. Analysis indicates that *RHD6* is a transcription factor preferentially expressed in root hair cells and is involved in the early stages of hair initiation. We have shown that in non hair cells *RHD6* is negatively regulated by *WER*, *TTG* and *GL2* and is positively regulated in hair cells by *CPC*. Data also idicates *RHD6* may have a negative self regulatory role.

253 Analysis of bHLH (MYC) genes involved in root hair and trichome differentiation

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Analysis of *R*, a maize bHLH (MYC) gene, in *Arabidopsis* strongly suggested that bHLH gene(s) regulate the differentiation of root hairs and trichomes [Lloyd et al. (1994), Science 266: 436]. It is also reported that *Arabidopsis GLABRA3* (*GL3*) encodes a bHLH gene [Payne et al. (2000), Genetics 156: 1349]. To analyze bHLH genes for epidermal cell differentiation, we are investigating the *GL3* homologues, *AtMYC2*, *AtMYC1*, *TT8*, as well as *GL3*. From the analysis of promoter::GUS plants, *GL3*, *AtMYC2*, and *AtMYC1* were strongly expressed in root-hair cell files, but *TT8* was not in root at all. In the shoot, *GL3*, *AtMYC2*, *AtMYC1* were similarly expressed in early stage of the leaf primordia. However, their expression patterns became different from each other as the leaf grew. We isolated *atmyc1-27660*, *atmyc1-10900*, and *atmyc2-5712* mutants from the T-DNA insertion lines of Kazusa DNA Research Institute. We found that *gl3-1*, *gl3-2*, *atmyc1-27660*, and *atmyc1-10900* had more root hairs than wild type at four days after incubation. *gl3-1 atmyc1-27660* and *gl3-1 atmyc2-5712* had more root hairs than each parental line. In addition, *35S::GL3*, *35S::AtMYC2*, and *35S::AtMYC1* plants showed the reduced number of root hairs. For trichome formation, *gl3-1*, *gl3-2*, and *atmyc1-27660* showed the reduced number of trichomes, whereas *atmyc2-5712* did not have an obvious phenotype. *gl3-1 atmyc2-5712*, however, had no trichomes at all. These results indicate that *AtMYC1*, *AtMYC2*, and *GL3* cooperatively regulate epidermal cell differentiation. We are now analyzing expression patterns of the three bHLH genes in root-hair and trichome mutants such as *cpc*, *try*, *gl2*, and *ttg1*, and also analyzing those of *GL2* and *CPC* in *gl3*, *atmyc2*, and *atmyc1* mutants. We will discuss genetic interactions of the bHLH genes with other regulatory genes for epidermal cell differentiation.

254 Role of *TRANSPARENT TESTA GLABRA2* in Root Epidermal Patterning

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The specification of root hair and non-hair epidermal cell types is positionally regulated in *Arabidopsis* and represents an attractive model system for studying cell patterning. Several genes have been identified as regulators of root epidermal patterning. *GLABRA2 (GL2)*, a homeodomain protein, has been identified as a positive regulator of the non-hair cell fate. *WEREWOLF (WER)*, a myb-related protein, acts as a positive regulator of *GL2* and thus the non-hair cell fate. *TRIPTYCHON* and *CAPRICE*, both encoding single repeat myb-related proteins, act as negative regulators of *GL2* and the non-hair cell fate. Moreover, a close relationship has been demonstrated between genes that regulate root epidermal and leaf trichome cell fates. Recently, *TRANSPARENT TESTA GLABRA2 (TTG2)*, a WRKY type transcription factor, has been identified as a regulator of leaf trichome development and seed coat mucilage production (Johnson *et al*., 2002). Interestingly, *TTG2* expression in the atrichoblast cells of the root epidermis has been shown, however, the role of this gene in root epidermal patterning is unknown. In further characterization of the role of *TTG2* in the root epidermis, we have identified additional mutant alleles. We are investigating the relationship of *TTG2* with other known components of root epidermal patterning.Our results confirm the involvement of *TTG2* in root epidermal specification.

255 Genetic analysis of MADS domain regulatory factors expressed in embryos.

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Members of the MADS domain family function as important developmental regulators; however, little is known about what roles they play during the embryonic phase of the life cycle. Through expression analyses, we learned that transcripts corresponding to at least 31 different members of the MADS domain family accumulate in embryonic culture tissues. At least 5 family members, including the floral repressor FLC, appear to be expressed at higher levels in embryos than in seedlings or floral tissues. To learn more about what roles these factors might play, we have isolated lines carrying T-DNA insertions in a number of these genes. Viable homozygous mutants have been recovered in every case, suggesting that none of the MADS domain genes that we have examined to date is indispensable for embryogenesis. We are exploring the extent of genetic redundancy through a combination of expression analyses, generation of transgenic plants, and analysis of double mutants. Supported by USDA NRICGP 2001-35304-1088.

256 Characterisation of Novel Flowering Time Mutants

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The switch from vegetative growth to flowering is influenced by environmental cues in order for reproductive growth to coincide with favourable seasonal conditions. In Arabidopsis, the photoperiod pathway promotes flowering in response to long days, while many ecotypes also respond to a prolonged cold treatment, known as vernalization. The combined effect of these signals is to promote flowering in spring. Many Arabidopsis mutants with altered flowering time have been characterised, predominantly in early flowering, vernalization insensitive ecotypes. We have screened an activation tagged population of the late flowering, vernalization sensitive ecotype C24, in an attempt to isolate novel flowering time mutants not found in earlier loss-of-function screens. We have isolated a number of early flowering mutants. By determining expression levels of the vernalization responsive floral repressor, *FLC*, the photoperiod sensitive floral activator, *CO*, and the floral integrators *SOC1 AGL20* and *FT*, we are dissecting the molecular pathways affected in the mutants. For example, some mutants have reduced expression levels of *FLC*, in most cases concomitant with an increase in expression of the floral integrators *SOC1* and *FT*. Other early flowering mutants have apparently normal levels of *FLC* and the expression levels of other flowering genes are being assessed. This will allow us to classify mutants as being affected in the vernalization/autonomous, the photoperiod or the GA pathway to flowering. Segregation analyses and transgenic approaches will allow us to confirm the genetic lesion conferring early flowering on the tagged mutants. Progress in characterising these mutants will be presented.

257 Mapping *pse13*, a photoperiod sensitive suppressor of *elf3-1*

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The mechanism by which plants regulate flowering in response to photoperiod has attracted great interest due to the possibility of genetic manipulation of crop plants. Past research has identified a number of genes involved in the photoperiodic regulation of flowering, but it is clear that this pathway is poorly understood and additional genes must be discovered. To isolate new genes we have identified second-site modifiers of *elf3-1*, a null mutation that results in early flowering photoperiod insensitive plants. One such second-site modifier of *elf3-1*, *pse13* (*photoperiod-sensitive suppressor of elf3*), suppresses the *elf3-1* early flowering phenotype and restores photoperiod sensitivity. Preliminary data from linkage analysis experiments suggests that *PSE13* is tightly linked to *LUMINIDEPENDENS* (*LD*).

258 Analysis of the role of UFO by identifying its interacting factors

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UNUSUAL FLORAL ORGANS (UFO) is a positive regulator of *APETALA3 (AP3)* expression. In addition to this regulatory role, UFO also has additional roles in flower development, such as defining boundaries within floral primordia and controlling cell proliferation. *UFO* encodes a protein containing an F-box, which was shown to be responsible for the interaction with *Arabidopsis* Skp1 like proteins, ASK1 and ASK2*. Many F-box proteins are known to be components of a functional SCF complex (Skp1, Cullin, and F-box), a ubiquitin protein ligase (E3) that brings substrates to the complex and thereby targets them for degradation by the proteasome. However, some of F-box proteins form non-SCF complexes with a variety of proteins in vivo. To define the role of *UFO*, we are exploiting two different strategies: yeast two-hybrid screens and immunopurification of a protein complex including UFO.

In our yeast two-hybrid screen, ΔFUFO, which has a deletion in the F-box, was used as bait to facilitate the interaction with factors other than ASK1 and ASK2. A cDNA library from *Arabidopsis* inflorescences was screened for interactions. Over a half of the candidates (19/37) encode ubiquitin conjugating enzymes (E2s), suggesting that UFO has a role in targeting substrates for ubiquitination. In order to circumvent limitations of the yeast two-hybrid screen, we are trying to purify a protein complex containing UFO from transgenic plants that harbor either TAP (tandem affinity purification) or epitope tagged UFO. Transgenic plants containing tagged version of ΔFUFO are also being used for this purpose as well, since, in principle, ΔFUFO can prevent substrates from being degraded. Progress in identifying in vivo factors that copurify with UFO will be presented.

Reference:"* Samach A, et al, 1999, Plant Journal 20, 433-445

259 Gain-of-function mutants obtained by an EN-I transposon activation tagging approach resemble known loss-of-function mutants

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Activation tagging has been often used to identify novel phenotypes. We have used the En-I transposon based activation tagging approach to screen for flower mutants and 3 interesting dominant mutants were identified. They were designated *needle* (*ndl*), *downwards siliques* (*ds*) and *twisted* (*twt*). Interestingly, all three "activation" mutants show phenotypes that have been previously described for knockout mutants. The *ndl* mutant resembles the *pinoid* mutant, *ds* is similar to *brevipedicellus* and the phenotype of *twt* shows characteristics present in the *tornado* mutant. The overexpressed genes were located downstream of the EN-I transposable element, up to a distance of 5.0kb. To confirm that we had the right genes in hand, we overexpressed cDNA clones under the control of the 35S promoter. For 2 out of 3 genes, corresponding knockout mutants were obtained, however, no visible phenotypes were observed. Studies to elucidate the relationship between the gain-of-function mutants and the resembling loss-of-function mutants are in progress.

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260 CPK28: A calcium-dependent protein kinase essential for embryo development

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Calcium-dependent protein kinases (CDPKs) contain a serine/threonine protein kinase domain fused to a series of calcium-binding motifs. To date, CDPKs have been found only in plants, algae and protists. There are 34 different members of the CDPK family in *Arabidopsis*, and members of this gene family share extensive sequence homology, from ~30% to 95% amino acid identity. Biochemical studies on selected CDPKs have confirmed that calcium directly regulates their kinase activities. Therefore, CDPKs may serve as important sensors and effectors of calcium signals and modulators of various physiological processes (1, 2).

In order to better understand *in vivo* functions of the *CDPK* gene family, we have been systematically searching for *Arabidopsis CDPK* knockouts in large T-DNA transformed populations (3). Here we describe one of these *CDPK* knockouts (*cpk28-1*), in which heterozygous plants produce 25% aborted seeds following self-pollination. Homozygous mutant embryos fail to progress beyond the globular stage of development. This defect can be rescued through introduction of the wild type *CPK28* gene in trans. *CPK28* is therefore an essential gene in *Arabidopsis*. To our knowledge, this represents the first report of a dramatic phenotype caused by the disruption of a single *CDPK* gene in any system, and supports the notion that CDPKs are indeed important mediators of calcium signals.

We would like to further understand the role of CPK28 throughout plant development. Currently, we are using a combination of techniques to study the spatial and temporal localization of this essential protein, and to find potential substrates and/or interactors. Results of these experiments will shed further light on the importance of CPK28 and calcium signaling in the development of a multi-cellular embryo and perhaps other physiological processes.

(1) Hrabak, E. M et al. Plant Physiol (2003) in press.
(2) Cheng, S.-H. et al. Plant Physiol (2002) 129: 469-85.
(3) Krysan, P. J. et al. Plant Cell (1999) 11: 2283-2290.

261 Analysis of the flowering time regulator, fsu2 (FRI suppressor 2)

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The late-flowering trait of Arabidopsis winter annual ecotype is conferred mainly by two genes; *FRIGIDA (FRI)* and *FLOWERING LOCUS C (FLC)*. To further elucidate the genetic control of flowering, we have screened FRI suppressor mutant by activation-tagging mutagenesis. In this study, one early-flowering mutant, *fsu2* (FRI suppressor 2), we isolated. *Fsu2* mutant was semidominant. *Fsu2* mutant showed slightly vernalization sensitivity. T-DNA was inserted in the second intron of novel MADS box gene *FSU2A*). *FSU2A* showed highest similarity to *FSU2B*, which located next to *FSU2A*. The amino acid sequences of *FSU2A* and *FSU2B* showed high similarity to AGL20. Both *FSU2A* and *FSU2B* genes are overexpressed in *fsu2* mutant.

262 Identification and Characterization of LEAFY Transcriptional Targets in the Floral Transition in *Arabidopsis*

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The switch from vegetative development to reproductive development has been shown to be mediated by the transcription factor LEAFY (LFY). However, only one direct target gene of LFY is known, *APETALA1*, that acts to control this developmental transition (Wagner et al., Science, 1999; Parcy et al., Nature 1998). Genetic analyses indicate that additional direct targets of LFY exist that regulate the onset of reproduction in *Arabidopsis*. Using a post-translationally activatable form of LFY, 35S::LFY-GR, we sought to identify these unknown LFY targets in *Arabidopsis* seedlings. Whole genome *Arabidopsis* microarrays were probed with cRNA from control treated seedlings or from seedlings treated with dexamethasone to activate LFY-GR. We also activated LFY-GR in the presence of protein synthesis inhibitor. Using stringent selection criteria, 17 candidate targets of LFY were identified by genome wide analysis and confirmed using quantitative RT-PCR from independently treated seedlings. We limited further analyses to the 5 most highly induced candidate LFY-target genes. In order to test the efficiency of the microarray-based approach at identification of direct LFY targets, we chose to investigate the biological role of the putative target genes during the switch to reproductive development. Towards this end we identified and are characterizing loss-of-function mutations in the candidate genes. Two parallel approaches were employed: T-DNA insertion mutants were identified from available collections and RNAi expression knockdown experiments were performed. Analysis of the loss-of-function mutant phenotypes and of the expression levels of the candidate genes will be presented.

263 Characterization of a family of Arabidopsis single C2H2 zinc finger proteins

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The zinc finger protein gene family is the largest group of transcription factors, and its members are involved in diverse and very significant roles in growth and development. Higher plants contain several distinct types of zinc finger proteins not found in other eukaryotes, one of which contains only a single Cys2-His2 (C2H2) zinc finger domain. The best characterized member of this family is the *SUPERMAN* gene that is required for flower and ovule development. Expression of ten additional genes of this family has been documented (Tague and Goodman, 1995, Plant Mol. Biol. 28: 267; Dinkins et al., 2002 Plant Cell Physiol 43:743). However, the function of these proteins in development is unknown. The expression profiles for other single zinc finger genes have been done in our laboratory, and we will present these results. Analysis of T-DNA insertion lines into the coding region of the single zinc finger genes uncovered one line that lacks petals and has reduced fertility, suggesting that this gene also plays an important role in flower development as well as ovule development. Analysis is ongoing to verify that the T-DNA insertion is the cause of the observed phenotype. T-DNA insertions into several other single C2H2 zinc finger genes have not displayed any abnormal phenotype suggesting that these genes may be redundant or non-essential. Ectopic expression of three of the single zinc finger genes in tobacco and Arabidopsis resulted in abnormal plants that were dwarfed, had abnormal leaf morphology, flowered early and most of the plants were sterile. Thus, ectopic expression of these transcription factors severely disrupts development. To further characterize the functional domains of two of these proteins, a GFP fusion protein with N-terminal portions of the AtZFP11 protein showed that the amino acids adjacent to the zinc finger domain determine the nuclear localization. Deletion of the leucine rich region similar to other ERF transcriptional repression domains at the carboxyl terminus, and fusion of the GFP protein adjacent to the putative ERF domain, results in transgenic plants that were phenotypically no different from wild type plants.

264 *BRACTS* is necessary and sufficient for bract development

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In *Arabidopsis thaliana*, major architectural changes take place during floral transition and affect many aspects of shoot development. One change, which unites *Arabidopsis* with the other members of the Brassicaceae family, is the suppression of leaf development in the inflorescence. We identified an Activation-tagged mutant termed *bracts-1D (brx-1D)* in which these leaves (bracts) develop on the inflorescence of *Arabidopsis*. The *BRX* gene belongs to a family of single C_2H_2 zinc-finger transcription factors in plants with a close homologue, *BRACTS-LIKE*, in *Arabidopsis*. Loss-of-function mutant analysis and RNAi studies show that *BRX* and *BRL* are required for the proper formation of all organs in *Arabidopsis*. Additional gain-of-function studies show *BRX* to be a floral repressor whose activity is limited by *AP1* and *FUL* in the flower and Inflorescence, respectively. Apart from this activity, *BRX* also has potent affects on morphogenesis of flowers and leaves when missexpressed and is able to suppress organ boundaries and activate promiscuous growth.

265 Photothermal Regulation of Flowering Time: A Mathematical Model

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The mechanism of flowering time control in *Arabidopsis thaliana* has been revealed by molecular genetic studies. To incorporate this knowledge and the effects of environmental factors on flowering time into a mathematical model, we have conducted a series of growth chamber experiments with seven flowering time mutants in the Ler background under several combinations of growth temperature (16, 20, 24°C) and photoperiod (8 and 16 hours daylength). Based on a qualitative, multi-pathway gene regulatory network model we developed a dynamic differential equation model to describe the accumulation of regulatory gene mRNA and protein levels and to predict flowering time. The model accounts for 88% of observed variation in data for eight genotypes. The model also simulates the circadian rhythmic expression pattern of most clock-regulated genes in the photoperiod pathway. The simulated gene expression patterns are compared with observed data from various data sources. These results demonstrate phenotype prediction from gene regulatory information. Because of the conservation of gene regulatory networks in plant species, the model might be modified and applied to agricultural relevant crops.

266 Germination in Arabidopsis Pollen: Polarization and Emergence of the Pollen Tube

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In germinating *Arabidopsis* pollen, a pollen tube emerges from the grain precisely at its point of contact with the stigma, breaching first the exine wall and then the papillar cell wall. This pollen-stigma contact point is distinguished by a lipid- and protein-containing "foot," which rapidly provides the pollen cell with a focused source of stigma water within minutes of the grain?s arrival. We are investigating the early polarization of the pollen tube relative to this spatial cue, together with the mechanisms necessary for tube exit directly through the exine, in contrast to exit through an aperture. We have found that many pollen tubes emerging from mutant *cer6-2* pollen (pollen lacking key lipid and protein coat components, and thus forming a deficient foot) are unable to identify the point of contact with the stigma and wander aimlessly, as do tubes emerging from wild-type pollen hydrated from all sides by placement in a high humidity chamber. Several minutes of focused water reception are sufficient to appropriately polarize the tube. In addition to using confocal laser scanning and electron microscopes to characterize the discrete, early cytoplasmic rearrangements accompanying *Arabidopsis* germination, we are gathering evidence for and against exine-breaking mechanisms based upon internal turgor pressure, secreted gel swelling, and exine weakening (both from the interior and exterior of the pollen grain).

267 The role of gibberellins, sugars and the autonomous pathway in the control

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The control of flowering in Arabidopsis under non inductive SD conditions is controlled mainly by genes in the Gibberellin (GA) and the autonomous pathways. GA is required for Arabidopsis flowering under short day conditions and they promote flower initiation by activating the promoter of the flower meristem-identity gene LFY. The activation of the LFY promoter by GA is rapid and does not require synthesis of any knew proteins by translation. During vegetative growth LFY is expressed in leaf primordia and very young leaves and under short days LFY expression increases gradually until the first flower is formed. This led us to study the developmental changes in the metabolism of GA in apical tissue during growth under short days. The increased LFY expression is paralleled by an increase of floral active GAs preceding the formation of the first flowers. We also analyzed the metabolic changes in soluble sugars and as for GA there is an increase in the sucrose levels before flowering. To determine whether the increase in sugars before flowering is important for the timing of flowering we have look for how genes involved in regulation of flowering time are affected by application of different sugars analogs. Autonomous pathway mutants flowers late in SD due to an increase in the expression of the strong flower repressor FLC. One possible factor which could be controlled by FLC is the GA pathway. To find this out have we analyzed whether the expression of GA metabolic enzymes or the sensitivity to GA were affected in SF2-FRI plants which contain high levels of FLC. We also analysed of how the expression of flowering time genes changes during growth in SD in wild type and SF2-FRI plants. From this have we been able to get a broader understanding on how the different genes interact in the control of flowering time.

268 Mapping of the *elf3* suppressor *PSE1*

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The study of photoperiodic responses in *Arabidopsis thaliana* has resulted in the discovery of numerous genes that regulate the initiation of flowering. However, it is evident that not all of the genes involved in photoperiodic regulation have been discovered. We have been using secondary mutant screens to isolate novel genes that are involved in flowering. The *ELF3* gene regulates flowering and the *elf3-1* null allele results in a loss of photoperiod sensitivity. We have mutated *elf3-1* seed and isolated a series of mutations that suppress the *elf3-1* phenotype, restoring wild type flowering. *photoperiod-sensitive suppressor of elf3* 1 (*pse1*) shows the strongest phenotype of these secondary mutations. Current mapping data for *PSE1* suggests that it is located at the top of chromosome 4, between the markers nga8 and JV32/33.

269 Methods to identify *in vivo* target genes in Arabidopsis

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Several large families of transcription factors exist in plants, among them the MADS-box gene family, which comprises a little over 100 members. In plants they are involved in e.g. flowering, flower formation, fruit dehiscence, reproduction, and leaf and root development. MADS-box proteins do not only form dimers, but are also capable to form ternary and quaternary protein complexes in yeast. They bind as complexes to motifs in promoter sequences of target genes, called CarG-boxes ($CC(A/T)_6GG$). To date only a few target genes from MADS-box genes have been identified. Our aim is to isolate target genes of AGAMOUS, SEPALLATA 3 and FRUITFULL, which are involved in pistil and silique development. Methods to identify *in vivo* target genes are: protein fusions with the glucocorticoid receptor in combination with micro array experiments, Chromatin Immuno Precipitation (ChIP) or Chromatin Affinity Purification (ChAP). ChIP allows purification of *in vivo* formed complexes of a DNA-binding protein and associated DNA. ChAP also allows isolation of protein-DNA complexes, but is based on the purification of epitope-tags that are fused to the protein of interest. For the ChAP approach an expression vector is made with a Strep-tag II and a FLAG-tag. The advantage of ChAP is that the same antibodies can be used to identify target genes from different transcription factors. The latest results will be presented.

270 Isolation of mutants, unable to establish the bilateral symmetry in the apical portion of *Arabidopsis* embryo

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In dicotyledonous plants, the embryo develops two cotyledon primordia and the shoot apical meristem (SAM) from its apical region after the globular stage. Region between cotyledon primordia become boundaries, where the growth is suppressed so that the two cotyledon primordia are separated each other. Bilateral symmetry is presented in the apical region of the embryo due to the symmetrical positioning of the cotyledon primordia and boundaries. Previous studies used *Brassica juncea* showed the phytohormone auxin is essential for the establishment of bilateral symmetry. Our previous studies also clarify that auxin functions during *Arabidopsis* embryogenesis as in *Brassica juncea* and that the mutation in the *PIN1* (*PIN-FORMED1*), encodes an expected auxin efflux carrier, causes almost completely the defects of bilateral symmetry in the apical region. In *pin1* mutant, the expression patterns of marker genes, such as *FIL* (*FILAMENTOUS FLOWER*), *CUC1* (*CUP-SHAPED COTYLEDON1*), and *CUC2* genes showing bilateral symmetry in the wild-type embryo, exhibit no bilateral symmetry but only radial symmetry in the apical region of the transition-stage embryos. To understand the molecular mechanism of the establishment of bilateral symmetry in the apical region of the globular-stage embryo, we tried to isolate the essential genes like the *PIN1* gene. We carried out a screen for the genetic enhancer of *pid* (*pinoid*). The *PID* gene encodes a protein-serine/threonine and is involved in the apical patterning during embryogenesis. The screening focused on mutant loci exhibiting the suppressed cotyledon development as seedlings of *pin1 pid* double mutant. Several enhancers exhibiting suppressed cotyledon development were identified in the *pid* background, while they showed the defects of cotyledon separation and number at a quite low frequency without the mutation of the *PID* gene. Now, we are trying to clone 4 genes of them by map-based cloning and analyzing their role in establishing the bilateral symmetry.

271 Delayed maternal-to-zygotic transition in maize

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In animals, early embryogenesis is dependent on maternal transcripts deposited in the egg cell before fertilization: the zygotic genome becomes active only after several rounds of embryo cell division. Similarly, genetic analysis in *Arabidopsis* has suggested that early seed development does not require paternally expressed genes. Maternal transcripts are thought to be sufficient to direct the initial development of both the embryo and the endosperm. However, it is not known whether these maternal transcripts are gametophytic (deposited prior to fertilization), zygotic (produced after fertilization), or both. We used a combination of expression profiling tools to answer this question in maize, which provides appropriate tools not necessarily found in *Arabidopsis*. Our data suggests that in maize, as in animals, the maternally controlled gametophytic phase extends several days beyond the first divisions of the zygote. They indicate that the early stages of embryo development are mainly or totally dependant on deposited transcripts, and that the maternal-to-zygotic transition takes place at least 3 days after the initiation of embryo development.

272 Regulation of pollen tube growth by a ROP GTPase signaling network in Arabidopsis

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Pollen tubes expand by tip growth and extend directionally toward the ovule to deliver sperms during pollination. In vitro pollen tubes display oscillatory growth and therefore provide an excellent model system for the study of temporal and spatial coordination of polar growth. We have shown that ROP1, a pollen-specific member of the plant-specific Rop subfamily of Rho GTPases, is a central regulator of pollen tube tip growth. Our earlier results suggest that the dynamics of tip F-actin, which is dependent on the localization of ROP1 GTPase to the apical region of pollen tubes, is required for polar growth in pollen tubes. Our studies also suggest that ROP1 regulate both the assembly of tip actin and the formation of tip-focused Ca2+ gradient. We have identified two functionally distinct ROP targets. Overexpression of one target protein promotes the actin polymerization in the tip of pollen tube whereas loss-of-function mutant cause hypersensitivity to Latrunculin B treatment. These data indicate this target protein promote actin assembly. Another target protein appears to be involved in the establishment of tip focused calcium gradient. Evidence suggests that these two target proteins together are required for actin dynamics. We propose that ROP1 regulate F-actin dynamics through these two distinct downstream pathways that interact with each other in a spatially and temporally coordinate manner and the temporal and spatial actin dynamics is a central mechanism for the control of oscillatory pollen tip growth.

273 SECRET AGENT Plays a Role in Flowering Time

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In plants there are two genes that encode O-linked N-Acetylglucosamine transferase (OGT) proteins, *SECRET AGENT(SEC)* and *SPINDLY(SPY)*. Mammalian OGT is a cytoplasmic enzyme, which adds an O-linked N-Acetylglucosamine (O-GlcNAc) residue to serine or threonine amino acids of a wide variety of phosphoproteins found in the nucleus, nuclear pore and cytoplasm. In some cases, O-GlcNAc modification and phosphorylation compete for the same serine/threonine residues. In arabidopsis, mutations in both *SEC* and *SPY* are gametophyte- and embryo-lethal indicating that these proteins have overlapping roles in plant reproductive development. Current work seeks to define the overlapping vs. unique roles of *SEC* and *SPY*. Previous studies have shown that *SPY* is a negative regulator of gibberellin signal transduction. Recent results will be presented demonstrating that *SEC* has a role in determining the number of days until flowering.

274 Genomics approaches towards understanding the vernalization response

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Many late-flowering ecotypes and mutants of Arabidopsis flower early following an extended cold treatment or vernalization. Vernalization down-regulates the expression of the MADS box protein FLC (a repressor of flowering). The maintenance of this down-regulated state is dependent on the action of the VRN1 and VRN2 proteins and sequences in intron 1 of the FLC gene. Our aim is to find more genes involved in mediating the vernalization response in order to understand the underlying molecular mechanisms. One approach we have taken is to analyse changes in the transcriptome in vernalized plants using microarrays representing over 11 000 Arabidopsis genes. Data from these experiments will be presented. The general conclusion from a large number of experiments is that vernalization leads to relatively few changes in the abundances of mRNAs that can be detected by microarray. We are also taking a mutagenic approach using the C24 ecotype. C24 has a high level of FLC expression and a strong response to vernalization. We have screened around 25 000 T-DNA tagged lines for either activation tags or knockouts and identified a number of mutants that have an altered vernalization response.

275 Analysis of BEL1 function in *Arabidopsis* ovule development using a steroid induction system

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Ovule development in *Arabidopsis* provides a simple system to study pattern formation and morphogenesis. In the developing ovule, there is formation of three morphologically distinct proximal-distal pattern elements. The central patterning element gives rise to the integuments, which are protective structures that grow asymmetrically to enclose the nucellus, and which later differentiate into the seed coat. Previously, we showed that the gene encoding the BEL1 homeodomain protein is required for integument formation and is expressed in the central patterning element of developing ovules. We have constructed a steroid-inducible system to regulate both the amount and timing of BEL1 activity in developing ovules. We demonstrate that early induction of BEL1 activity restores normal ovule development in *bel1-1* mutants. Interestingly, when BEL1 activity is induced late in ovule development, integument formation is restored but growth is incorrectly polarized. We are currently testing how BEL1 regulates genes known to control polarity in the ovule. These findings will help to define a role for BEL1 in ovule patterning and in determination of integument identity.

276 Identification of Proteins that Interact with AGAMOUS-Like 15 (AGL15), a MADS-Domain Transcription Factor that Preferentially Accumulates in the Plant Embryo

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AGAMOUS-Like 15 (*AGL15*) is 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. Some of these target genes are induced in response to AGL15, while others are repressed. AGL15 itself does not appear to possess an activation domain, which poses the question of how it is able to activate the transcription of its downstream target genes. A number of target genes have been analyzed that bind AGL15 and exhibit strong responses to AGL15 levels *in vivo*, yet *in vitro*, AGL15 binds only weakly. Taken together these data suggest 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 screen an embryo expression library for putative interactors of both full length AGL15 and a truncated form lacking the MADS domain. Proteins partners capable of interacting with AGL15 in the yeast two hybrid assays will be presented.

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277 Function of two biotin carboxyl-carrier protein-genes in gametophytes and ovules of *Arabidopsis*

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Heteromeric acetyl-CoA carboxylase catalyzes the first and committing reaction of de novo fatty acid synthesis in plastids. This enzyme is composed of 4 subunits, biotin carboxyl-carrier protein (BCCP), biotin carboxylase (BC), alpha-carboxyltransferase (alpha-CT) and beta-carboxyltransferase (beta-CT). In Arabidopsis, single-copy genes encode BC and CT. Two BCCP-coding genes (*CAC1A*(At5g16390) codes for BCCP-1; *CAC1B* (At5g15530) codes for BCCP-2) are both expressed. In situ hybridizations indicate that both*CAC1A* and *CAC1B* mRNAs accumulate throughout maternal tissue of siliques (1 DAF), and developing embryos. Accumulation of *CAC1B* mRNA is maximal in globular embryos, in contrast, *CAC1A* accumulates maximally during embryo growth and triacylglyceride deposition. We were unable to recover homozygous mutant cac1a plants among progeny from heterozygous T-DNA-tagged (*CAC1A/cac1a*)plants, indicating the *CAC1A* gene is essential for seed development. Also, lack of *CAC1A* function reduces transmission of the male gamete. In contrast, T-DNA-tagged mutations in the*CAC1B* gene have no apparent affect on plant development. To identify the basis for the effects of the *cac1a* mutation, gametophytes and post-pollination development of the embryo were examined in heterozygous (*CAC1A/cac1a*) plants. Ovule development appears normal, however, exine patterning is uneven and pollen tube growth is altered. Cellular abnormalities can be detected as early as 2-cell embryo stage. Mutant embryos develop more slowly than WT, and are arrested by the heart stage; subsequently the embryo sac collapses. Endosperm cellularization does not occur, and endosperm degenerates. Thus, although both genes are expressed during reproductive development,*CAC1A* function is essential and cannot be substituted by *CAC1B*, whereas *CAC1B* function is not required for normal reproductive or vegetative development.

278 A mutation in the *TILTED* locus affects the placement of the root pole during embryogenesis and post-embryonic root growth

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One of the events that occurs during early plant embryogenesis is the correct placement of the prospective shoot and root poles at the opposite ends of the embryo. Little is known about how this happens. We have found a mutant, *tilted*, in which at least part of this process is affected. Homozygous mutant embryos are delayed in their development with respect to their wild type siblings, taking longer to proceed through the globular stage. Morphological abnormalities are seen first during the globular stages and mostly involve abnormal patterns of division in the hypophyseal cell and its derivatives. This leads to a root pole that is not aligned with the top of the suspensor, and a "tilted" appearance for the embryo. Abnormal cell divisions are sometimes observed for the hypocotyl region, but do not lead to abnormalities later in development. Root and shoot pole markers have been used to corroborate these observations. After germination, the mutant roots grow more slowly than wild type roots, and are somewhat insensitive to the inhibition of growth by auxin. *TILTED* is also necessary for ovule development, but we have not yet fully investigated this aspect of the phenotype. We are further characterizing the mutant phenotypes and finely mapping the affected gene. Our current data suggest that *TILTED* has a role in embryo patterning and possibly a role in post-embryonic root growth.

279 KOMPEITO is important for callose accumulation and exine formation in *Arabidopsis* microgametogenesis and is localized in golgi apparatus.

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Plant reproduction begins with the adhesion of pollen grains to the female stigma. We previously showed that the morphology of the outermost layer of the pollen grains, the exine layer, is important for this adhesion step, using *kompeito* mutant that is defective in the exine sculpture. However, the molecular mechanism of the formation of the exine sculpture is not well understood.

The defect in *kom* was first observed during meiosis stage. In *kom*, no callosic staining was found around the pollen mother cell. In tetrad stage, callose wall was observed, but the amount of callose was much smaller than wild type. At the same stage, sporopollenin was randomly deposited onto the plasma membrane of the microspore, and following exine formation was disrupted.

KOM gene was isolated by map-based cloning and it encoded a seven-path transmembrane protein. *KOM* was expressed in pollen mother cell during meiosis stage. Using a GFP fusion protein, we investigated the subcellular localization of KOM protein. The dot-like fluorescence of KOM-GFP fusion protein was not merged with the fluorescence marked by FM4-64, an endosome marker. The fluorescence of KOM-GFP fusion protein was altered by BFA treatment, suggesting that KOM is localized in golgi apparatus.

Based on our results, it is suggested that callose wall is important for the formation of the exine layer, and that *KOM* is essential for the meiosis stage-specific callose accumulation. A possible role of KOM protein for microsporogenesis will be discussed.

280 Genetic Dissection of Autonomous Flowering Pathway in Arabidopsis

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Flowering of Arabidopsis is promoted by at least three interacting genetic pathways; photoperiod, vernalization and autonomous pathways. In the autonomous pathway, *FLOWERING LOCUS C*(*FLC*) is known as a central molecule acting as a floral repressor. *FLC*is positively regulated by *FRI*, while it is negatively regulated by the autonomous pathway and vernalization. From fast neutron mutagenesis of *FRI*-Col, a very late flowering line, we isolated mutants that flower as early as Columbia ecotype. The genetic complementation showed that at least 14 mutants are novel, which we named them as *suf*(suppressor of *FRI*). *sufs* were analyzed for the response to photoperiods and vernalization. Three suf mutants, *suf9, 12, 14* flowered earlier than Col in SD and showed hypersensitivity to continuous red(Rc) light for hypocotyl elongation. In contrast, another *suf* mutant, *suf8* was insensitive to Rc for hypocotyl elongation. *suf3* mutant flowered earlier than Col in SD only when *suf3* was vernalized. The molecular analysis of sufs showed that *suf3* and *suf13* were acting between *FLC* and *AGL20* but others are acting between *FRI* and *FLC* in genetic hierarchical pathway. The map-based gene cloning of *suf*s are in progress.

281 Characterisation of progamic phase mutations in Arabidopsis

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The progamic phase of reproductive development involves post-pollination events from pollen germination to gamete fusion. As a strategy for the identification of gametophytic genes that function during the progamic phase we have used transposon insertional mutagenesis based on screening for distorted segregation of an antibiotic resistance marker. From a screen of 3, 616 Ds transposon insertion lines, we isolated 19 independent lines showing strongly reduced segregation ratios arising from reduced gametophytic transmission. Among these, we identified 9 male-specific (seth) mutations that could be divided into two classes according to their transmission efficiency (TE): seth1-6 (TE < 1%) and seth7-9 (TE < 25%). seth lines produced pollen with normal cellular morphology and showed no ovule failure or seed abortion, suggesting progamic defects excluding fertilisation events. The low penetrance of seth7-9 suggested that these mutations had a quantitative effect on pollen germination and tube growth. Indeed, the male transmission of seth7-9 was restored in non-competitive pollinations, indicating that mutant pollen tubes are able to reach the micropyle and fertilize, but less efficiently than their wild type counterparts. In contrast, the transmission of only one strongly penetrant mutations (seth6) was partially restored in non-competitive pollinations. Cytological analysis showed that seth1-6 mutations act early, reducing pollen germination efficiency and/or pollen tube growth. The SETH genes include both pollen-specific and constituvely expressed genes involved in biological functions as diverse as cell wall biosynthesis, glycosylphosphatidylinositol anchor synthesis, protein phosphorylation and metabolism. Four SETH genes encode novel proteins whose function in plants or in other systems remains unknown. Progress in molecular and phenotypic analysis will be presented.

282 Functional Specificity of the AP3/PI MADS Box Transcription Factors

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The *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) genes are necessary for petal and stamen development in the flower. They encode members of the plant MADS box family of transcription factors and are more closely related to each other than to other MADS box proteins in plants. AP3 and PI act as obligate heterodimers and presumably exert their organ identity functions through transcriptional regulation of downstream genes. The plant MADS box proteins are characterised by the MADS DNA binding domain, an intervening region (the I box), an additional region of homology, the K box, and divergent C-termini. Despite the relatively high sequence similarity between the MADS box proteins involved in floral development and their similar *in vitro* DNA binding preferences, these proteins must regulate different downstream targets to produce the different floral organs. One possible mechanism for generating specificity among these proteins is by the recruitment of distinct cofactors, which would help to refine their target specificity. Since the C-termini of these proteins are the most divergent in sequence, these regions are possible targets for cofactor binding. Comparison of *AP3* and *PI* orthologues from a wide range of species has identified short highly conserved motifs within the C-termini of both AP3 and PI. A series of truncation and domain swap constructs have been generated and expressed in Arabidopsis to assess the importance of these motifs. Our analyses suggest that these motifs are necessary for the full function of AP3 and PI, and that the AP3 and PI C-termini have distinct functions. Additionally, the C-terminus of AP3, when fused in frame to PI, is sufficient to allow AP3 function. These results suggest that the evolution of different C-terminal motifs was a critical component of the functional diversification of these gene lineages and suggest that factors important for functional specificity of these MADS box proteins may bind to these motifs. To identify these putative cofactors involved in petal and stamen development, two- and three-hybrid screens using the C-termini of AP3 and PI as bait are currently underway in the lab. Preliminary results of these screens will be presented.

283 A mechanism related to the yeast Paf1 transcriptional regulator is required for activation of *FLOWERING LOCUS C* expression

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The Arabidopsis *VERNALIZATION INDEPENDENCE (VIP)* genes regulate multiple developmental processes, including timing of flowering and floral development. In flowering, they are strong activators of *FLOWERING LOCUS C (FLC)*, a transcriptional switch used to prevent flowering until after an extended period of cold (vernalization). Seven *VIP* loci have currently been identified. Genetic and molecular epistasis experiments suggest that the *VIP* genes act as components of a common mechanism. *VIP4* encodes a protein closely related to a component of the Paf1 complex, a yeast transcriptional regulator that assists in the targeting of a Srb/Med-independent form of RNA Pol II to a small subset of genes. To better understand how the *VIP* mechanism regulates its target genes, we cloned *VIP5* and *VIP6*. Both genes encode proteins related to additional subunits of the Paf1 complex. Thus, the *VIP* genes may represent a plant form of the Paf1 complex in which the VIP proteins may act together in a transcriptional protein complex to regulate a subset of genes including *FLC* and floral development genes. One possibility is that this regulator is recruited to *FLC* or an upstream *FLC* activator by a vernalization-regulated transcription factor. In additional developmental contexts, alternative transcription factors could convey temporal or spatial information and target the regulator to other developmentally important genes.

284 MAP-kinase signaling in the early Arabidopsis embryo

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Upon fertilization, the Arabidopsis zygote elongates more than 2-fold and divides transversely into a small apical and a large basal cell. The daughter cells of the zygote subsequently establish two lineages with different developmental fates. Cells of the basal lineage continue to elongate and to divide transversely producing the mostly extra-embryonic suspensor. Cells of the apical lineage switch to a more isodiametric growth and produce the pro-embryo. We have identified mutations in three genes that affect this process in a similar way, and have characterized one of them, designated YODA, in detail. Mutant zygotes elongate only about 30% before dividing. While development of the apical cell is initially not affected, cells of the basal lineage are much smaller than in wild type and their division pattern is irregular. No visible suspensor is formed, and a molecular marker for suspensor development is absent in 80% of the mutants. Despite this early defect, YODA embryos can occasionally develop all pattern elements and grow into viable seedlings. The YODA gene encodes a MAPKK kinase expressed throughout development in all tissues analyzed. All 9 alleles we recovered are predicted to abolish or reduce kinase activity. Manipulations in the presumptive regulatory domains of the protein result in artificial gain-of-function alleles which have opposite phenotypic effects as loss-of-function alleles. Growth of the suspensor is exaggerated, and growth of the pro-embryo is suppressed. In the most severe cases, the zygote develops into a file of cells, as if development of the apical cell was arrested, or all cells had assumed a suspensor identity. We propose that YODA identifies a MAP-kinase signaling pathway that modulates a fundamental cell-fate decision in the early embryo. YODA promotes a developmental program characterized by elongation, transverse division, and, ultimately, extra-embryonic fate. After the asymmetric division of the zygote, YODA is down-regulated in the apical lineage which becomes free to form the pro-embryo.

285 Analysis of the flowering network using mutations in *SOC1*, a floral pathway integrator in Arabidopsis

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Flowering in Arabidopsis is regulated by photoperiod, autonomous, and vernalization pathways. A MADS box protein, SOC1 has been recently characterized and was shown to integrate these pathways. In this study, we further analyzed the role of SOC1 in the flowering network by generating double mutants with late flowering mutants. Double mutants of *soc1* null with late flowering mutants in the photoperiod pathway showed additive effects while *soc1* effect was masked in double mutants with autonomous pathway mutants. This result indicates autonomous pathway is the major pathway regulating *SOC1*. However, *soc1-101D* (activation-tagged mutant) further suppressed the late flowering of photoperiod mutants than that of autonomous pathway mutants. In addition, *ft soc1-101D* flowered similar to *fca soc1-101D* or *FRI soc1-101D*. Examination of *FT* expressions in the late flowering mutants suggested that *FT* expression level is the key factor in regulating flowering time in an *SOC1* overexpression background. Vernalization of the double mutants of *soc1* with autonomous pathway mutants showed that *soc1* mutation dampened the vernalization response in the single mutants. This suggests that SOC1 has an important function in mediating the vernalization signals for flowering. We also show that *SOC1* functions partially downstream of *FT* and upstream of *LFY* by analyzing the expressions of the flowering pathway integrators in *soc1-101D* and *35S::FT*.

286 Functional characterization of EMF-mediated floral repression

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Flowering time in higher plants is finely regulated by floral inducers and floral repressors. Recent investigation of the early flowering mutants in Arabidopsis reveals a variety of mechanisms by which flowering can be delayed and vegetative growth extended (Sung et al., 2003). While some early flowering genes interfere with the signaling process or inhibit the expression of floral inducer such as the flowering time gene, *FT*, several genes such as *CURLY LEAF1* (*CLF1*) and the *EMBRYONIC FLOWER* (*EMF*) genes appear to delay flowering by repressing the flower organ identity genes directly. *EMF1* encodes a nuclear protein that represses the expression of flower organ and the seed maturation genes (Moon et al., 2003). EMF2, a polycomb group (PcG) protein, is also nuclear-localized and represses the flower organ genes probably via the formation of protein complexes. To investigate the molecular mechanism of EMF-mediated floral repression, we have been studying *EMF* expression, and the proteins that interact with EMF. The progress of our research will be reported.

Moon et al., 2003. EMF genes maintain vegetative development by repressing the flower program in Arabidopsis. Plant Cell 15: 681-693.
Sung et al., 2003. Mechanisms of floral repression in Arabidopsis. Curr. Opinion in Plant Biol. 6: 29-35.

287 Regulation of the flower-specific expression of *AtSUC1*

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The Arabidopsis thaliana *AtSUC1* gene encodes a sucrose carrier protein and is exclusively expressed in flowers. Expression occurs in the connective tissue of anthers during anther dehiscence, in mature pollen grains, in a ring under the stylar tissue, in the surface cell layer of the septum (placenta) and in funiculi. Different types of regulation are used for *AtSUC1* expression in different tissues: For example, translational control is used in pollen grains, where *AtSUC1* mRNA is synthesized in the pollen grain already in anthers. However, translation of *AtSUC1* mRNA occurs not prior to hydration of the pollen on the papillae of the stylar tissue. In contrast transcriptional control of the *AtSUC1* gene occurs in the funiculi and the placenta where *AtSUC1* transcription and translation are co-induced by pollination. After pollination a strong spatial and temporal relation between the length of the growing pollen tube and the *AtSUC1* expression in different female organs was found. We used the *AtSUC1* promoter in combination with the *GFP* reporter-gene for deletion analyses *in planta*. Only the smallest fragment (-235 bp) resulted in a complete lack of GFP fluorescence. In further experiments gel retardation assays with nuclear extracts of freshly pollinated flowers were accomplished. Two different regions in the promoter were identified as possible interaction sites for transcription factors. These regions are currently being analysed in more detail using smaller fragments for gelshift assays. The obtained sequence data will be used for one hybrid screens to identify interacting transcription factors.

288 Control of Seed Size by AP2

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Seeds from plants with strong mutant alleles of *APETELA2* (*ap2-6*, *ap2-7* and *ap2-11*) are significantly larger than those of wild type. The reduced fertility of *ap2* mutants due to its abnormal flower structure does not account for the larger size of mutant seeds. *ap2* mutant mature embryos and dry seeds seem to be morphologically normal except for the abnormal seed coat (Jofuku et al., [1994] Plant Cell **6:** 1211-1225) and the large seed size. As compared to wild type, protein levels in *ap2* mutant seeds are uniformly higher than wild type, in proportion to the seed weight.

The *ap2* mutation affects seed size through at least two distinct mechanisms. First, *ap2* mutant embryos grow larger than wild type after the torpedo-stage. By the bent cotyledon-stage, *ap2* mutant embryos are significantly larger than wild type. *ap2* mature embryos have more cells and larger cells than wild type embryos. Extension of seed maturation concomitant with the prolonged mitotic activity of developing embryos accounts for the extended growth of *ap2* mutant embryos. Second, the *ap2* mutation affects seed cavity size. When the cavity of wild type seeds reaches its maximal size at the mature green-stage, *ap2* mutant seeds are still at the bent cotyledon-stage and have a larger seed cavity. The large cavity of developing seeds may allow *ap2* mutant embryos to undergo extended growth. The *ap2* mutation affects all these distinctive features of seed development maternally to make big seeds.

We thank Drs. Diane Jofuku and Jack Okamuro of Ceres Inc. who independently discovered the large seed phenotype of *ap2* mutants for their advice and discussion.

289 Determination of Functional Significance of *FCL* Splicing Variants

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FLC is the central repressor of the floral transition in *Arabidopsis* . *FLC* is highly expressed in plants with a dominant allele of *FRIGIDA*, and can be down-regulated by the autonomous floral promotion pathway and vernalization treatment. Five *FLC* homologues have been identified based on their DNA sequence similarity, named *FLM* and *FCL1-4*. Supported by phenotypes of gain-of-function and/or loss-of-function mutants, these *FLC* homologues are suggested to be floral repressors similar to *FLC*.

For three of these homologues (*FLM*, *FCL3*, and *FCL4*), at least two alternative splicing products have been detected, but only one may encode a protein homologous to the floral repressor FLC. In the case of *FCL3* and *FCL4*, the other splicing variants retain the third intron and introduce a premature stop codon shortly after MADS domain. Previous experiments showed that the FLC-like type of splicing products of these *FLC* homologues, when overexpressed, might lead to a delay of flowering phenotype similar to that caused by overexpressing *FLC*. However, our data suggest that the I3-retaining variant is the predominant form for *FCL3* and *FCL4*. We propose to study the functional significance of this splicing variant by gain-of-function analyses.

The redundancy and/or overlapping of function of these *FLC* homologues are also of interest to us. It has been shown that *FLC* knockout mutants can still respond to vernalization. The *FLC* homologues have been proposed as candidates of this *FLC*-independent vernalization response. We plan to investigate this residual vernalization effect in double knockout mutants, i.e. *fcl4 flc*. We also suggest that the regulation of *FCL4* by vernalization could be at both transcriptional and post-transcriptional levels. *FCL1* and *FCL2* may repress the floral transition in response to other environmental cues, which will be investigated in the knockout plants.

290 Structural and Functional Analysis of the LEAFY COTYLEDON1 Transcription Complex

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LEAFY COTYLEDON1 (LEC1) is a central regulator of embryogenesis that controls embryo identity in Arabidopsis. LEC1 is required for the specification of cotyledon identity, acquisition of desiccation tolerance, maintenance of suspensor cell fate, and inhibition of precocious germination. *LEC1* expression is normally limited to embryogenesis, but when ectopically expressed, *LEC1* induces embryonic programs and somatic embryo formation from vegetative cells. Thus, LEC1 establishes a cellular environment that promotes embryogenesis. The LEC1 protein shows significant sequence similarity with the HAP3 subunit of the CCAAT binding transcription factor (CBF). Arabidopsis has gene families encoding homologs of the three subunits, HAP2, HAP3, and HAP5, found in yeast and mammalian CBFs, suggesting that plants have functional CBFs.

To understand the molecular mechanism by which LEC1 regulates embryogenesis, we are determining how LEC1 influences the activity of the CBF complex. Specifically, we would like to know if the CBF complex containing LEC1 acts differently than complexes containing other Arabidopsis HAP3 subunits that are not embryonic regulators. To this end, we investigated CBF complex formation both *in vivo* and *in vitro*. We will discuss the ability of Arabidopsis HAP subunits, including LEC1, to form CBF complexes and the activities of these complexes.

291 An Exotic Analysis Project for Determining Gene Expression and Function in *Arabidopsis thaliana* (Landsberg *erecta*).

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EXOTIC (EXOn Trapping Insertion Consortium) is an EU-funded project involving nine European laboratories with the aim of determining the function and expression of *Arabidopsis* genes on a large-scale basis. About 17,500 *Ds*-GUS-tagged *Arabidopsis* (L*er*.) lines have been produced within the consortium. Approximately 2,500 lines represent in-frame insertions and show GUS expression. Analyses of the lines include characterization of GUS expression patterns in different tissues at various stages as well as forward and reverse genetic screens for specific biological functions. A database containing sequence and GUS expression data is currently being generated (www.jic.bbsrc.ac.uk/hosting/exotic/). The database will be publicly accessible at the end of the project (autumn 2003). In our part of the consortium we are focusing on lines showing tissue specific expression and lines that are either early or late flowering compared to wild type. In an initial screening of 8,000 lines for flowering time, we identified 47 lines, which flowered earlier than wild type and 33 lines that flowered later than wild type. The first early flowering lines have been confirmed by re-screening and these are now in progress of being further characterized. Several lines with specific expression patterns in various tissues have been identified and the insertion sites of the *Ds* element were identified by TAIL-PCR. Promoters of the corresponding genes are currently being analysed for regulatory elements.

292 Characterization of AERIAL ROSETTE 1 (ART1) flowering gene

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The genetic changes underlying the diversification of plant forms represent a key question in understanding the plant macroevolution. To understand the mechanisms leading to novel plant morphologies we investigated the Sy-0 ecotype of Arabidopsis that forms an enlarged basal rosette of leaves, develops aerial rosettes in the axils of cauline leaves and exhibits inflorescence and floral reversion. We showed that this heterochronic shift in reproductive development of all shoot meristems results from synergystic activation of FLOWERING LOCUS C (FLC) by AERIAL ROSETTE 1 (ART1) and FRIGIDA (FRI) (1). ART1, is a new flowering gene that maps 14cM proximal to FLC on chromosome V. Progress in its cloning will be reported. In addition, the Sy-0 morphology can be phenocopied in gi ART1 FLC and FWA ART1 FLC plants, suggesting that the reduced expression of floral pathway integrators (FPI: FT, SOC1 and LFY) underlie this phenotype. These results demonstrate that modulation in flowering-time genes is one of the mechanisms leading to morphological novelties.

(1) Poduska B, Humphrey T, Redweik A, Grbic V (2003) The Synergistic Activation of FLOWERING LOCUS C by FRIGIDA and a New Flowering Gene AERIAL ROSETTE 1 Underlies a Novel Morphology in Arabidopsis. Genetics 163,1457-1465.

293 Unraveling the cascade of gene activation during fruit development

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The development of the fruit of *Arabidopsis thaliana*, from its inception to the dehiscence or releasing of the seeds, is a complex and genetically coordinated series of events. Mutations in several transcription factors have revealed their important roles during fruit development. Among them, are three MADS box proteins, FUL, SHP1, SHP2, two basic helix-loop-helix, IND and ALC, and a recently incorporated homeodomain protein, RPL. Although these proteins are required to form the different parts of the mature fruit, little is known about the targets of these transcription factors. With the goal of shedding some light on the cascade of transcriptional activation triggered by these key genes, we have generated inducible versions for most of them by making GR (glucocorticoid receptor) fusions. The transgenic lines incorporating these constructs are being used to study gene expression profiles by taking advantage of microarray technology. Here, we report the initial characterization of the different transgenic lines, the setup of the induction experiments and some preliminary results about the transcriptional targets of FUL and IND that we have found using this approach.

294 The role of prenylated proteins in floral organ patterning

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Arabidposis flowers typify an evolutionary trend among flowering plants toward fixed flower organ numbers and positions. This includes a propensity toward alternations of organ positions, such that inner whorl organs arise directly between outer whorl organs. Many Arabidopsis mutants that affect flower patterning do not affect alternation of organ position, suggesting the presence of a signal or signals that cross whorl boundaries to specify the position of organ initiation. We have isolated mutants in the PLURIPETALA (PLP) gene, which we propose may be involved in this process. Flowers of plp mutants show a slight increase in the average numbers of sepals and carpels, but show a dramatic increase in petal and, occasionally, stamen number. Moreover, the positions of petals and stamens are unrelated to positions of other floral organs. PLP encodes a key protein required for protein prenylation, which is a lipid post-translational modification that typically serves to facilitate membrane association of signaling molecules. Like era1, a previously identified prenlyation component, plp mutants affect other aspects of plant development, such as shoot meristem size and hormone signaling, but the two mutants show some differences, with plp mutants generally showing additional and more severe phenotypes. Double mutant studies suggest that PLP acts in the same pathway as ERA1, and also suggest roles for prenylation-mediated signaling in additional aspects of leaf and flower development. We are currently focusing on identifying and determining the roles of plp target proteins in flower organ patterning.

295 Characterization of an *Arabidopsis* Meiotic Mutant Line Produced by an Unusual T-DNA Insertion Event

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Morphological characterization of fertility-impaired mutant lines produced by T-DNA mutagenesis, identified a line that produces a limited amount of pollen exhibiting aberrant exine patterning. Backcrossing this pollen onto emasculated WT plants is able to effect fertilization, although with a lower rate of success than that achieved by WT selfing. WT pollen backcrossed onto the mutant line produces viable seed in normal quantities. Light microscopic observation of semi-thin (0.5µm) sections of anthers indicate that meiosis does not proceed normally, resulting in coenocytic microsporocytes containing varying numbers and sizes of "nuclei." Prophase spreads of mutant microsporocytes have been performed to confirm that chromatids are not correctly apportioned between daughter nuclei.

Plasmid rescue was performed on the line. Analysis of the resulting clones revealed that portions of the *Agrobacterium tumefaciens tra* regulon appear to have been incorporated into the plant during the insertion event. No flanking plant DNA was observed in these clones. A genomic Lambda library was therefore constructed and screened using the *tra* fragment farthest from the T-DNA border in the plasmid rescue clone, along with normal left and right border T-DNA probes. Analysis of twenty-five lambda clones isolated in this screening is currently underway to more fully characterize the structure of the T-DNA insertion and adjacent plant DNA.

296 *grv2*: A viable embryonic mutant with a putative role in endocytosis

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The *grv2* mutant was identified in a screen in Arabidopsis for mutants with altered morphology at the globular stage of embryogenesis. The *grv2* mutant is characterized by enlarged cells in the apex of the embryo, first observed at the two-cell stage in cleared whole mount specimens. The enlarged cells appear to have a single nucleus with an abnormally large vacuole. The embryo recovers by the late heart stage at which time the enlarged cells are no longer observed. Despite this early embryonic phenotype, the *grv2* mutant develops into a relatively normal adult plant except for a reduction in the growth response to light and gravity. *GRV2* encodes a large gene with homology to *RME8*, a *C. elegans* gene involved in endocytosis.

297 LEUNIG and SEUSS Putative Co-repressors in AGAMOUS Regulation

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In *Arabidopsis*, proper spatial and temporal expression of homeotic genes is essential for the development of flowers. Precise regulation of floral C class homeotic gene AGAMOUS (AG) is critical to flower pattern formation. In wild type, AG expression is limited to the inner two whorls of flower and is required for specification of stamen and carpel identity. However in *Leunig* (*lug*) and *Seuss* (*seu*) mutants, AG is ectopically expressed in the outer two whorls. In addition, double mutants of *lug* and *seu* exhibit enhanced homeotic transformation of whorls 1 and 2 organs due to ectopic and precocius AG expression. This suggests that *lug* and *seu* are involved in the negative regulation of AG in whorls 1 and 2 of flowers. LUG is a nuclear protein that has a domain structure similar to transcriptional co-repressors Tup1 of yeast and Groucho of *Drosophila* which have Q-rich regions at the N-terminus and 6-7 WD repeats at the C-terminus. In addition, the first 88 aa of LUG defines another highly conserved domain named LUFS domain, whose function is not known. Similarly SEU is a novel protein with two Q-rich domains and a highly conserved dimerization domain showing similarity to LIM-domain binding transcriptional co-regulators in animals. Since both LUG and SEU are involved in negative regulation of AG, we tested physical interactions between LUG and SEU. LUG and SEU interacted in the yeast two hybrid assay, and the N-terminal LUFS domain in LUG is necessary and sufficient for the interaction with SEU. In contrast SEU requires both the Q-rich and the dimerization domains to interact with LUG. At present, we are confirming LUG and SEU interaction using in-vitro protein association assay, as well as in vivo assay using transgenic plants. To determine the repressor function of LUG and SEU, both proteins were tethered to the promoters of reporter genes through the Gal4 DNA-binding domain. LUG can repress reporter gene transcription in both yeast and plants. In contrast, SEU cannot. Based on these results, we propose a model in which, LUG and SEU interacts physically and forms a co-repressor complex that might be recruited by DNA-binding transcriptional factors to the AG cis-regulatory elements.

298 Transcriptional regulation of NAC genes by the floral homeotic genes AP3/PI

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In Arabidopsis, the floral homeotic proteins APETALA3/PISTILLATA act as a heterodimer and are necessary for petal and stamen development. To gain insight into how AP3 and PI act to regulate organ identity we have performed a screen for indirect and direct targets using a system where AP3/PI function is put under posttranslational control. Differential screening using the YALE 9.2k Arabidopsis EST microarray identified genes belonging to the NAC gene family as being targets of the AP3/PI heterodimer. Apart from *NAP* (NAC-LIKE, activated by AP3/PI) we identified a previously uncharacterized NAC gene as being negatively regulated by AP3/PI. Northern blot experiments confirmed these microarray results and also showed that other NAC family members are also negatively regulated by AP3/PI. Taken together our data suggests that these genes may act downstream of AP3/PI during flower development, possibly in a redundant manner.

299 Molecular and genetic analysis of the REM gene family

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The REM gene family is a moderately-sized gene family that is distantly related to several classes of known plant DNA binding proteins, including the B3 class of proteins such as VP1 from maize and ABI3 from Arabidopsis, the auxin response factors, and the RAV proteins. So far the function of only one REM gene has been determined (*REM39/VRN1*). The name of this gene family derives from the reproductive meristem (REM)-specific expression patterns of the first members of the gene family to be characterized. There are 44 REM genes in the Arabidopsis genome. Although REM family members are present in other plants (e.g. rice and Brassica) there are no bacterial, fungal, or animal homologs of the REM genes. Based on this similarity, we postulate that the REM genes encode plant specific DNA binding proteins. One unusual feature is that the REM genes are clustered in the genome; nine REM genes (REM1-REM9) are clustered on chromosome 4, five REM genes (REM10-REM14) are clustered on chromosome 2 and three REM genes (REM40-REM42) are clustered on chromosome 5. The remaining REM genes are not clustered and are present on all five Arabidopsis chromosomes. We present our progress on the molecular and genetic analysis of the REM gene family.

300 A Genome-Wide Survey of Stigma-Expressed Genes in Arabidopsis

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In plants with dry stigmas, the stigma surface provides a platform for pollen adhesion, regulated hydration of the bound desiccated pollen, germination and orientation of the growing pollen tube. Much of the stigma machinery involved in these processes remains uncharacterized. The immediate goal of this research is to identify factors expressed by the stigma that may play a role in pollination using cDNA subtraction and microarray analysis. Stigma and ovary tissue, as well as seedling tissue, were collected from Arabidopsis thaliana by micro-dissection, and the RNA extracted. Multiple microarray replicates were then performed using the Affymetrix ATH1 genome array chip. The replicates were then statistically collapsed to single genome profiles. These profiles were then compared to each other to identify genes upregulated in stigma compared to ovary as well as genes upregulated in pistil compared to seedling. A cDNA subtraction experiment was performed on the stigma to verify in a different but complementary approach the stigma-specific expression profile. Over 1000 stigma enriched cDNA sequences were cloned and sequenced. The data from the two methods, when compared, illustrates biases inherent in both methods. Genes implicated in stigma function; including aquaporins, kinases, and lipid transfer proteins were among the most abundant in the stigma when compared to the ovary. Taken together, these experiments define a set of stigma-expressed genes that will serve as the basis for future functional studies.

301 TERMINAL FLOWER2, an HP1-like protein of Arabidopsis, counteracts transcriptional activation of a flowering gene, *FT* in vascular tissues of leaves

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In Arabidopsis, the competence of flowering is acquired in accordance with gradual increase of expression levels of flowering pathway integrators such as *FT*, *LFY*, and *SOC1*. To understand the molecular mechanisms underlying the regulation of flowering genes in the photoperiod pathway, we have analyzed Arabidopsis *terminal flower2* (*tfl2*) mutant, which exhibits a day-length independent early-flowering phenotype. We have previously shown that *TFL2* encodes a nuclear protein homologous to Heterochromatin Protein1 (HP1) and that *TFL2* functions as an epigenetic repressor that negatively regulates *FT* expression. To understand how levels of *FT* mRNA are regulated by TFL2, we investigated the effect of *TFL2* on *FT* activation by CONSTANS (CO), a direct transcriptional activator. By using promoter::GUS transgenic plants, we found that *CO*, *FT*, and *TFL2* are all detected in vascular tissues of leaves, and unexpectedly, *FT* was not detected in the meristem. In *tfl2* mutants, *pFT::GUS* expresion was upregulated within vascular tissues of leaves, suggesting that *TFL2* can reduce *FT* expression level against the CO activity. On the other hand, overexpression of *CO* (*35S::CO*) can derepress *FT* expression even in the presence of *TFL2*, although expression levels were reduced by *TFL2*, to some extent. Taken together, these results suggest that TFL2 cannot completely inhibit but can alleviate the effect of CO on transcriptional activation of *FT*.

302 Identifying Direct Downstream Target Genes of the Embryo MADS-domain Protein AGL15: an *in vitro* and *in vivo* Study

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AGL15 (for AGAMOUS-Like 15) is currently the only reported member of the plant MADS-domain family of transcriptional regulators that preferentially accumulates during embryo development. Additionally, AGL15 is one of the more divergent members of the MADS-domain family, including within the DNA-binding domain. Previous studies have shown that MADS-domain proteins bind to DNA sequences with an overall consensus of $CC[A/T]_6GG$ (called a CArG motif). Nonetheless, different MADS-domain proteins exhibit similar, yet distinct binding site preferences that may be critical for differential gene regulation. To determine the consensus sequence preferentially bound by AGL15 *in vitro*, PCR-assisted binding site selection assays were performed. AGL15 was observed to prefer a CArG motif with a longer A/T-rich core ($C[A/T]_8G$), and is to date the only plant MADS-domain protein having such a preference. We searched the *Arabidopsis thaliana* genome for genes containing AGL15 binding sites as candidates for direct regulation by AGL15. Here we report one gene, *DTA4* (for *Downstream Target of AGL15 4*), identified by this method. We confirmed that *DTA4* is a direct *in vivo* target of AGL15 by the following tests: 1) chromatin immunoprecipitation enrichment tests demonstrating a direct *in vivo* interaction between AGL15 and *cis*-elements within *DTA4*; 2) tests to verify that *DTA4* expression responds to AGL15 levels; and 3) transient reporter assays to show that the CArG motif of *DTA4* could confer regulation by AGL15. Our approach could be used to identify direct targets of other transcription factors.

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303 Ectopic expression of the FUS3 gene in the epidermis confers a heterochronic shift during vegetative development

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fusca3 (*fus3-3*) was isolated in a screen for Arabidopsis mutants that could germinate prematurely. This mutant accumulates less seed storage proteins in the embryo and is desiccation intolerant. Moreover, this mutant also accumulates anthocyanins and ectopic trichomes on the cotyledon. Because the mutant cotyledon partly resembles wild-type vegetative leaves, the *FUS3* gene is thought to be an important regulator of cotyledon identity. Promoter-GUS analysis shows that *FUS3* is expressed strongly in the protodermal layer during embryogenesis. This led us to hypothesize that targeted expression of *FUS3* in the epidermal layer is enough to complement the *fus3-3* mutation. To test this, we constructed transgenic lines in which *FUS3* expression is driven by the AtML1 promoter, an epidermal specific promoter. All known *fus3-3* mutant phenotypes were rescued by the transgene, including seed desiccation intolerance, vivipary and ectopic trichome formation. Localization studies of the FUS3-GFP fusion protein driven by the AtML1 promoter suggest that the fusion protein does not move from the epidermis. Because the *AtML1p-FUS3* transgene complemented not only epidermal layer associated phenotypes (trichomes) but also vivipary, seed storage protein accumulation, and seed desiccation tolerance, it is hypothesized that a downstream target of *FUS3* moves towards the inner cells as a cell-non autonomous signal. In addition, epidermal specific *FUS3* overexpressing lines showed a heterochronic shift during vegetative leaf development. We will discuss a possible role of the *FUS3* gene in plant development.

304 The Arabidopsis SeedGenes Project

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The purpose of the Arabidopsis SeedGenes Project is to coordinate the collection, analysis, and presentation of information on essential, non-redundant genes that give a seed phenotype when disrupted by mutation. Arabidopsis appears to contain ~750 such *EMB* genes required for seed development. Our long-term goal is to help the community establish a complete collection of Arabidopsis genes with a mutant seed phenotype. This information is needed to focus attention on genes with important cellular functions and to assess from a genetic perspective the extent of functional redundancy in the Arabidopsis genome.

The SeedGenes Project database (www.seedgenes.org) is organized into two major sections. One section deals with genes and the other with mutant alleles. The database can be queried for detailed information on a single gene to create a SeedGenes Profile. Queries can also generate lists of genes or mutants that fit specified criteria. The third database release (June 2003) contains information on ~220 essential genes and ~310 mutants. Included are many genes identified at Syngenta and all known genes described in the literature. The goal over the next two years is to present information on a total of 500 mutants defective in 300 *EMB* genes. Future enhancements include the addition of Nomarski images of mutant seed phenotypes, a tutorial on screening for seed defects, and information on genes expressed in young seeds.

Forward genetics will not enable the identification of every *EMB* gene through random mutagenesis. Small genes in particular will be difficult to detect. We are therefore using a bioinformatics-based approach to identify promising candidates for missing *EMB* genes based on sequence comparisons with essential genes in other model organisms. Candidate genes with evidence of seed expression and limited functional redundancy will then be subjected to reverse genetics using available insertion lines and screened for seed phenotypes.

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305 Investigating the Role of GABA in Pollen Tube Guidance in *Arabidopsis thaliana*

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The POP2 gene in *Arabidopsis thaliana* encodes a transaminase that modulates γ-amino butyric acid (GABA) levels. GABA is elevated 100-fold in *pop2-1*, resulting in a pollen tube guidance defect and sterility. In wild type ovules GABA is concentrated in the cells surrounding the egg, while in *pop2-1* it is abundant throughout the ovary, indicating a role for GABA in guiding the pollen tube to the female gametophyte. To further clarify the role of GABA in fertilization, I am generating mutants deficient in GABA synthesis in order to test the hypothesis that GABA is required for pollen tube guidance. I am also conducting a suppressor screen of *pop2-1* both on soil and on media containing 10 mM GABA, a concentration at which *pop2-1* seedlings show sensitivity to environmental GABA.

306 Phenotypic analysis of hapless mutants with disrupted pollen tube guidance.

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Plant reproduction is a precisely regulated, multi-step process involving complex cell-cell interactions. To achieve fertilization the male gametophyte, the pollen grain, must deliver its sperm cells to the female gametophyte, the embryo sac. Accomplishing this task requires that pollen successfully undergo a series of complex steps including recognition of a receptive female, polar cell growth and migration, and targeted cell fusion. To identify and characterize haploid-expressed genes required for reproduction in Arabidopsis, we have adopted T-DNA based gene tagging strategy with the expectation that disruption of an essential haploid-expressed gene will result in reduced transmission of the gamete and thus any marker genes on the T-DNA. In addition to a drug resistance gene, the construct carries a histochemical marker expressed only in mutant pollen grains and tubes. This novel approach allows for rapid in vivo assessment of pollen tube (PT) morphology, growth and targeting. ~ 10,000 independent transgenic T2 lines have been screened and 32 hapless mutants identified. Light microscopy was used to place mutants into one of four phenotypic classes: 1) pollen development, 2) pollen germination and early PT growth, 3) PT growth and guidance and 4) no apparent pollen defect. Of particular interest to us are hapless mutants with defects in PT guidance to the female gametophyte or entrance through the micropyle. Over one-third of the hapless mutants identified show evidence of a late stage PT growth or guidance defect, and reciprocal crosses indicate the vast majority specifically affect the male gametophyte. Using a combination of light microscopy, confocal laser scanning microscopy and in vitro tube growth assays, we have set out to finely characterize the PT phenotypes of hapless mutants defective in the final stages of pollination. Multiple interactions between pollen and the female reproductive tissue are necessary to for final stages of sperm cell delivery, yet little is known about the signals exchanged between male and female gametophytes. The fine-scale phenotypic analysis we have undertaken, combined with identification of the corresponding hapless genes, will provide a better understanding of the molecular mechanisms controlling specific stages of PT guidance to the ovule and into the micropyle.

307 Identification and functional analysis of *DTA1* (*downstream target 1 of AGL15-1*)

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AGL15 is currently the only identified member of the Arabidopsis MADS-box family that is preferentially expressed during embryogenesis. *DTA1*, a downstream target of AGL15, was identified using a chromatin immunoprecipitation (ChIP) approach. Regulation of *DTA1* by AGL15 was demonstrated as follows. First, the expression level of *DTA1* was upregulated in *AGL15* overexpressing plants and downregulated in *agl15* developing seeds compared to wildtype Arabidopsis. Second, a DNA fragment containing a non-canonical CArG motif (CCAATTTAATGG) that is located in the *DTA1* regulatory region was enriched in independent ChIP DNA populations generated using AGL15 antiserum, indicating an *in vivo* interaction between AGL15 and the DNA fragment. Thirdly, a mutation of CC to TT within this CArG motif causes the loss of interaction with AGL15 *in vitro* in gel mobility shift assays. Furthermore, the same mutation changes expression of a *GUS* reporter gene in response to AGL15 *in vivo*, where reporter lines containing the regulatory region of *DTA1* drive *GUS* expression. *In vitro* enzyme assays showed that DTA1 has gibberellin 2-oxidase activity. Overexpression of this gene in Arabidopsis causes a GA deficient phenotype and alters endogenous GA levels accordingly. The phenotype of *dta1* plants is the converse of those overexpressing *DTA1* and includes altered seed germination attributes.

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308 Gene expression analysis of *Arabidopsis* flower development

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For a better understanding of *Arabidopsis* flower development we are currently generating an expression map for genes that are expressed only at certain time points during flower development or specifically in certain parts of the flower. These spatially and/or temporally regulated genes may play an important role in the regulatory processes that pattern the flower or in the differentiation of the various types of floral tissues. We have initiated our study by comparing the gene expression patterns of wild-type flowers with those of mutants that show homeotic transformations using microarrays. In these homeotic mutants certain types of floral organs are absent or are replaced by other types of organs. By combining the data sets obtained in these experiments we were able to identify groups of genes that are predicted to be specifically expressed or strongly enriched in one of the four different floral organs: sepals, petals, stamens and carpels. The organ-specific expression patterns of several of these genes were confirmed by in-situ hybridizations. Furthermore, the expression patterns of genes with known organ-specific expression were predicted correctly by the microarray experiments. In addition, we are trying to identify the target genes of several of the many transcription factors that have been implicated in flower development. For this, we have fused the coding regions of the factors with a fragment of the glucocorticoid receptor and express the resulting fusion proteins in plants. These fusion proteins can be specifically activated by treating the transgenic plants with a steroid hormone. This system allows us to do time course experiments and observe changes in gene expression that occur shortly after the activation of the transcription factors as well as later changes that are presumably downstream of the primary events. Data of these experiments will be presented.

309 Tapetal development and expression of the transcriptional regulator MALE STERILITY1 (MS1).

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The *Arabidopsis MALE STERILITY1 (MS1)* mutant appears as wild type except for shortened siliques, due to a failure of self-fertilisation because of a lack of viable pollen. Failure of pollen development commences immediately after meiosis around the stage of microspore release. At this stage the tapetum appears highly vacuolated compared to wildtype. The mutant can be rescued by manual cross fertilisation using viable pollen, since female fertility is unaffected.

The MS1 protein has homology to a group of transcription factors that contain a PHD-finger domain (1). These proteins appear to be highly conserved within this domain and are found in humans, yeast and higher plants. They are thought to regulate transcription by chromatin remodelling (2). We therefore believe that MS1 is a key sporophytic controlling factor for the processes of anther and pollen development.

MS1 is expressed at a low level, in a highly regulated developmental manner. Expression is seen in the tapetum, starting around the stage of microspore release. Once the tapetum has degraded no further expression is seen. We have constructed functional GFP::MS1 protein fusions and protein expression has been analysed by confocal microspcopy. These fusions have shown that the protein expression mirrors our observations of transcriptional regulation. The fusion protein is extremely transiently expressed immediately prior to microspore release and is typically only seen in one bud from a complete inflorescence. The protein is nuclear localised. This data will be presented and discussed in relation to the functional role of MS1 during male gametogenesis.

References:
(1) Wilson, Z. A., et al. (2001). "The *Arabidopsis MALE STERILITY1 (MS1)* gene is a transcriptional regulator of male gametogenesis, with homology to the PHD-finger family of transcription factors." Plant Journal 28: 27-39.
(2) Asaland, R., et al. (1995). "The PHD finger: implications for chromatin-mediated transcriptional regulation." Trends in Biotechnology 20: 56-59.

310 The Role of the K domain in Mediating the Formation of the AP3/PI/SEP Terna

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APETALA3 (AP3) and PISTILLATA (PI) are two floral MADS homeotic proteins required for petal and stamen development in Arabidopsis. The three closely related MADS proteins, SEPALLATA1 (SEP1), SEP2 and SEP3, specify petal, stamen and carpel organ identity together with AP3, PI, and AGAMOUS (AG). SEP3 has been demonstrated to interact with AP3/PI in a yeast three-hybrid assay (Honma and Goto, Nature 409, 525 (2001)). in vivo functional studies support the hypothesis that a SEP3/PI/AP3 complex specifies petal development in Arabidopsis. Little is known about the subdomains and amino acids that mediate interactions among these three MADS proteins. Here we report on novel interactions of the SEP proteins with AP3 and PI. The three SEP proteins interact similarly with PI, AP3 and AG in yeast two-hybrid assays. The region of PI important for the PI/SEP interaction includes the second and the third amphipathic alpha-helices in the K domain (K2 and K3). By contrast, the PI/AP3 interaction is mediated primarily by the first and second amphipathic alpha-helicies in the K domain (K1 and K2}. As the common motif critical for both the PI/AP3 and PI/SEP interactions, the second amphipathic alpha-helix in the K domain (K2) of PI is proposed to be the key motif mediating the formation of the AP3/PI/SEP ternary complex.

311 Identification of proteins that interact with the pleiotropic developmental regulators *VERNALIZATION INDEPENDENCE 3, 4*

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The Arabidopsis pleiotropic *vernalization independence* (*vip1-7*) group mutants define a mechanism involved in multiple developmental processes. *VIP3* encodes a protein consisting of almost entirely of WD motifs, suggesting that this protein participates in a protein complex. *VIP4* encodes a nuclear-localized protein with sequence homology to subunit of a yeast transcriptional complex, suggesting that this protein may be involved in an analogous plant transcriptional complex. Our recent results are consistent with the hypothesis that VIP3 and VIP4 function closely both with a 'core' mechanism and with temporally or spatially defined components that adapt this mechanism for specific developmental events. We are carrying out two complementary approaches to identify additional potential components of the VIP mechanism. Two-hybrid screening of an Arabidopsis meristem expression library using VIP4 as the bait resulted in the identification of seven proteins. A reversed-genetics approach has been initiated to study the function of these proteins. A tandem affinity purification (TAP) approach was also initiated using genomic copies of *VIP3* and *VIP4* fused with a His6-2XFLAG TAP tag.

312 Control of expression and autoregulation of *AGL15*, a member of the MADS-box family

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AGL15 is an *Arabidopsis thaliana* MADS domain regulatory factor that is preferentially expressed during embryogenesis and potentially plays an important role in embryo development. *AGL15* is also expressed at lower levels after germination. To gain insight in the regulation of the expression of *AGL15*, we have initiated characterization of regulatory regions of *AGL15*. To find the potential *cis* regulatory elements, a series of deletions of a previously characterized reporter construct (Fernandez *et al.*, 2000, Plant Cell 12: 183-197) were carried out to dissect the promoter region of *AGL15*. We have found potential positive and negative *cis* elements in the 5' regulatory region of *AGL15*. Interestingly, AGL15 may contribute to control of its own expression. When the reporter constructs were introduced into a transgenic background where *AGL15* is being constitutively expressed, less reporter activity was observed. However, a modified form of AGL15 that includes a strong transcriptional activation domain (the VP16 activation domain), causes increased and ectopic expression of the reporter construct. This feedback loop may be due to direct or indirect regulation of *AGL15*. To determine whether regulation by AGL15 may be direct, we tested DNA populations enriched for *in vivo* AGL15 binding sites for the presence of fragments that correspond to the regulatory regions of *AGL15*. Furthermore, when either one of the two potential AGL15 binding sites are mutated in the reporter construct, the reporter activity were increased. The results suggest that AGL15 may directly regulate its own expression.

313 Genetic analysis of IBA-response mutants reveal novel components in peroxisomal processes

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Auxin is an important plant hormone that influences virtually all aspects of plant growth and development. Despite auxin?s ubiquitous involvement in plant processes, we are only beginning to understand how auxin levels are regulated. There are at least two endogenous forms of auxin, indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), previously thought to only exist as a synthetic auxin. IBA appears to undergo a two-carbon elimination reaction similar to the one used in peroxisomal fatty acid beta-oxidation to yield IAA. To understand the roles of IBA in plants, we are using forward and reverse genetics to isolate IBA-response (ibr) mutants that display enhanced root elongation on inhibitory concentrations of IBA and yet maintain a wild-type phenotype on IAA. These mutants may have aberrations in genes involved in IBA conversion or perception. We have categorized these mutants into three classes based on their root and hypocotyl phenotypes under various hormone, sugar, and light conditions: 1) those with defects in peroxisome or fatty acid b-oxidation, which directly or indirectly disrupt IBA conversion to IAA, 2) those that have defects particularly in b-oxidation of IBA, and 3) those without defects in b-oxidation, which may be defective in IBA perception . Interestingly, some ibr mutants with defects in b-oxidation map to regions lacking any genes known to function in peroxisomal processes. These mutants may define novel components that have yet to be identified in other organisms.

314 Proteomic isolation of stress-related proteins in Arabidopsis

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Proteomics is a leading technology for the large-scale analysis of protein expression, post-translational modifications, and protein-protein interactions. With the completion of genome sequencing projects and development in analytical methods for protein characterization, proteomics has become a major field of functional genomics. It has extensively been applied to the global analysis of gene products in different tissues and in various physiological states of cells. Our research efforts have been made to integrate proteomics into the systematic and comprehensive investigation of biological processes in plants. We are interested in two sub-proteomes, nuclear/DNA binding proteome and membrane/secreted proteome. Nuclear/DNA binding proteome including transcription factors plays central roles in a broad range of cellular activities through regulating gene expression. Membrane/secreted proteome including receptors, channels, and secreted peptides is also of active molecules that regulate cell-cell interactions of developmental processes and responses to the environment, and initiate and modulate diverse signaling pathways. This presentation describes the methodological establishment for purification of nuclear/DNA binding proteome and membrane/secreted proteome, proteomic analysis of the isolated proteomes, and further functional studies using biochemical and molecular biological methods. With the technical challenges to be solved, the methods have successfully been set up for isolation of nuclear/DNA binding proteins and membrane/secreted proteins. We have analyzed the protein spots on 2D-gels of the isolated proteomes using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). We also identified proteins that are up- or down-regulated in response to abiotic and biotic stresses. Reproducible and substantial changes in the expression of proteins have been observed in response to the stress treatments and those proteins are being analyzed.

315 Cross-talks between brassinolides and auxin in Arabidopsis

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Brassinosteroids (BR) and auxin exert some similar physiological effects and a potential functional interaction between them has been suggested, but the mechanism for this interaction is not clear. We found that low concentration of brassinolide (BL), the most active BR in nature, promoted lateral root formation, particularly the initiation of lateral root primodia in Arabidopsis. This effect could be inhibited by auxin transport inhibitor N-(1-naphthyl) phthalamic acid (NPA). Furthermore, 1 nM BL enhanced the promotion of lateral root formation by low levels of auxin (1-20 nM). To further explore the mechanism by which BR interacts with auxin, we examined the effect of BR on the expression of the b-glucuronidase gene fused the synthetic auxin-inducible promoter (DR5::GUS). DR5::GUS expression in the margin of cotyledons and young leaves and the stele of primary roots was promoted by BL, but inhibited either by treatment with brassinazole (a BR biosynthesis inhibitor) or by a mutation in BRI1, which encoding a BR receptor. These results suggest that at least some BR actions are to regulate auxin transport, accumulation or cell sensitivity to auxin in Arabidopsis. The auxin transport assay in Arabidopsis root showed that 25 nM BL increased auxin acropetal transport. However, we did not find any promoting effect of BR on IAA biosynthesis. The investigation of BR effect on cell sensitivity to auxin is underway. *DR5::GUS*

316 RUB1 family members function in multiple hormone response pathways and regulate ethylene production in dark-grown seedlings

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Rub, a ubiquitin-like protein, attaches to the cullin subunit of the ubiquitin pathway E3 ligase called the SCF complex. *In vitro* work using the mammalian RUB1 ortholog, Nedd8, determined that SCF activity requires this modification. RUB1 conjugation in *Arabidopsis* has been linked with auxin signaling; AXR1 (Auxin Resistant 1) encodes one component of a heterodimeric enzyme that catalyzes formation of a thioester-linked RUB1 required for attachment to cullins. To determine the role AtRUB1 plays *in vivo*, PTGS (Post-Transcriptional Gene Silencing) was used to reduce the levels of the two closely related Rub proteins, RUB1 and RUB2. Both mRNA and protein were reduced in all lines characterized compared to controls. These lines exhibit a dwarfed phenotype, distinct from *axr1-12*, and seedling roots show decreased responses to auxin and ethylene. In contrast to *axr1-12*, dark-grown 35S::dsRUB1 seedlings exhibit a partial triple response, associated with ethylene response that is suppressed in the presence of AgNO3 or AVG, ethylene receptor or synthesis inhibitors, respectively. Dark-grown 35S::dsRUB1 seedlings produce 3-6 times more ethylene than wild-type lines. Dark-grown 35S::dsRUB1 lines have a slight photomorphogenic phenotype, similar to *axr1-12*, implicting these proteins in skotomorphogenesis. These data have revealed a role for the Rub pathway in ethylene and auxin response and a previously undescribe role for the Rub pathway in ethylene production in *Arabidopsis*.

317 The ABI3 gene is involved in auxin signaling and lateral root development

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Genetic screens have identified a number of genes that regulate abscisic acid (ABA) responsiveness in *Arabidopsis*. The ABI3 gene was initially isolated as an ABA-insensitive mutant, and was found to encode a seed-specific transcription factor. However, recent evidence has shown that it has functions outside of the seed. Based on germination in the presence of exogenous ABA, it was determined that ABI3 acts at or downstream of ERA1, which encodes for the B-subunit of a farnesyltransferase (Brady et al. 2003). Further characterization of the expression of ABI3, using the ABI3 promoter fused to GUS, demonstrates that ABI3 is expressed in lateral root primordia, and in correlation with the genetic relationship between ERA1 and ABI3, ABI3 expression is upregulated in an era1-2 mutant. Further analysis indicates that ABI3 is ABA and auxin-inducible in lateral root primordia and that a loss of function *abi3-6* allele has reduced lateral root responsiveness to both auxin and NPA. Additional evidence of ABI3's defective auxin response will be demonstrated using the synthetic auxin response element, DR5. An interaction between ABA and auxin at the level of germination, and an analysis of auxin responses in a variety of ABA signaling and biosynthetic mutants will also be presented.

318 Activation Tagged Phototropic Mutants

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Environmental conditions drive plant development. To survive, plants must be able to adapt to the ever-changing environment. Light represents one major environmental regulator of plant growth and development, mediating responses ranging from changes in gene expression to induction of flowering. Plant fitness is therefore intimately tied to their ability to respond properly to a variety of light cues. As one example, it has been shown that Arabidopsis seedlings mutant for one particularly important light sensing protein, phototropin 1 (phot1), exhibit dramatically reduced emergence and establishment properties under both greenhouse and field conditions (Galen, Huddle, and Liscum, see poster). Our laboratory is interested in altering light sensing or response capacity of Arabidopsis seedlings. One approach we are taking is to use ?activation tagging? to identify novel gain-of-function mutants. Two such mutants have been identified and partially characterized. The first, *map5* (modifier of *arf7/nph4* phenotypes 5) is a dominant mutant in the *nph4/arf7-1* background that appears to contain reduced levels of brassinolide (BR). The *map5* mutant is able to partially suppress both the aphototropic and agravitropic phenotypes conditioned by the *nph4-1* allele. Upon BR application *map5 nph4-1* double mutants recover nph4-1 atropic phenotypes. A second mutant, HH29, is phototropically hyper-responsive, while maintaining normal gravitropism. The recessive nature of the HH29 lesion suggests that the affected gene normally functions as a repressor of phototropism. These mutants provide important new insights into phototropic signal responses.

319 Characterization and analysis of genes implicated in auxin-mediated development and in IAA biosynthesis

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Indole-3-acetic acid (IAA) is involved in virtually every aspect of plant growth and development. To better understand the role of IAA in root development part of our lab focuses on mutants defective in lateral root formation. One such mutant, *alf4-1*, was isolated because of its greatly reduced number of lateral roots. The mutation blocks lateral root development at initiation and the defect is not rescued by IAA, an inducer of lateral roots. In addition, the *alf4-1* mutant is male sterile and has reduced cell expansion in shoot tissues. We positionally cloned *ALF4* and found that the mutation deletes a splice site within *ALF4*. We have now found additional alleles that cause a similar *alf4* mutant phenotype suggesting that *alf4-1* is a null allele. The *ALF4* gene, which is unique in Arabidopsis, encodes a protein with no obvious similarities to known proteins; however ESTs from other plants share significant homology with ALF4. To learn more about *ALF4* we have begun to characterize the *ALF4* expression pattern and determine the ALF4 protein's intracellular location using reporter constructs. In addition, plants designed to overexpress ALF4 have been made.

In a different approach we are examining the role of IAA biosynthesis in development. Previously, we cloned genes encoding the cytochrome P450s, CYP79B2 and CYP79B3, which catalyze the conversion of tryptophan to indole-3-acetaldoxime, a proposed intermediate in IAA biosynthesis[a]. Recently, we have found that CYP79B2 overexpression results in longer hypocotyls and elevated transcription of known auxin-induced genes. Moreover these overexpressors have a ~1.5-fold increase in auxin. Conversely, cyp79B2 cyp79B3 double mutants are more diminutive than wild-type plants and under certain conditions produce <70% of the wild-type level of auxin[b]. We are currently examining the role of the CYP79B2/3 pathway in plant development and its relationship to other IAA biosynthetic pathways and tryptophan metabolism.

[a]
Hull, A. K. et al. (2000). Proc. Natl. Acad. Sci. USA 97, 2379-2384.
[b]Zhao, Y.et al. (2002). Genes & Dev. 16, 3100-3112.

320 Post-translational Regulation of ACC Synthase in Ethylene Biosynthesis

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The Arabidopsis ethylene overproducing mutants *eto1*, *eto2* and *eto3* have been suggested to affect the posttranscriptional regulation of ACC synthase. The positional cloning of the gene corresponding to the dominant *eto3* mutation indicates that the *eto3* phenotype is the result of a missense mutation within the C-terminal domain of ACS9 which encodes one isoform of the Arabidopsis ACC synthase gene family. This mutation is analogous to the dominant *eto2* mutation that affects the C-terminal domain of the highly similar ACS5. Analysis of purified recombinant ACS5 protein and epitope-tagged ACS5 in transgenic Arabidopsis revealed that *eto2* does not elevate the specific activity of the enzyme either in vitro or in vivo, but rather increases the half-life of the protein. In a similar manner, cytokinin treatment increased the stability of ACS5, by a mechanism that is at least partially independent of the *eto2* mutation. The *eto1* mutation was found to act by increasing the function of ACS5 through a stabilization of this protein. These results suggest that an important mechanism by which ethylene biosynthesis is controlled is by the regulation of the stability of ACS protein, mediated at least in part through the C-terminal domain. The turnover of myc-ACS5 fusion protein is strongly inhibited by a specific inhibitor of the 26S proteosome and the high molecular weight complex (>600KDa) in addition to the monomer and dimer forms of myc-ACS5 were found by a size fractionation chromatography. Potential components of this complex and the interactors identified by ACS yeast two-hybrid screen will be discussed.

321 Characterization of ethylene receptor signaling complexes from the Arabidopsis endoplasmic reticulum

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A family of Arabidopsis ethylene receptors (ETR1, ERS1, ETR2, ERS2, EIN4) are related to bacterial two-component histidine kinases. CTR1, a downstream target of the receptors, is related to the Raf family of Ser/Thr MAPKKKs. Thus, the ethylene receptors and CTR1 represent a specific case in which a two-component pathway may directly feed into a MAPK cascade. However, the underlying mechanisms for such signal transduction have been unclear, although it has been proposed based on two-hybrid analysis that the ethylene receptors and CTR1 may physically associate (Clark et al., 1998). We determined by using sucrose density gradient centrifugation that ETR1 and ETR2 primarily localize to the endoplasmic reticulum (ER) of Arabidopsis. Interestingly, we also find that CTR1 is ER-associated. Localization of CTR1 to the ER could be mediated by interactions with the ethylene receptors. This hypothesis is supported by our finding that loss-of-function mutations in the ethylene receptors result in a redistribution of CTR1 from the membrane to the soluble fraction of Arabidopsis. A pulldown assay confirmed that a tagged version of CTR1 in plants co-purified with ETR1. When analyzed by gel filtration, we find that ETR1 isolated from plants is present in a signaling complex of 670 kDa. In contrast, ETR1 transgenically expressed in yeast fractionates by gel filtration as a dimer of 150 kDa. The size of the 670-kDa ETR1 signaling complex was not changed when analyzed in mutant backgrounds containing loss-of-function mutations in other members of the ethylene receptor family. Our results indicate that ETR1, CTR1, and other unknown factors form a stable complex of 670 kDa that functions in ethylene perception and signaling in planta.

322 Analysis of ABA insensitive mutants expressing random GFP:DNA fusions

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The plant hormone abscisic acid (ABA) has a key role in regulating seed dormancy and germination. Generally two types of genetic screens have been employed to dissect the signaling pathway of this response. These involve screening for germination mutants that are either ABA-insensitive (*abi*) or have an enhanced response to ABA (*era*). Most mutations affecting ABA sensitivity in germination have been identified in the Columbia ecotype and are genetically recessive; only two dominant mutations have been identified to date. These dominant mutations are in the Landsberg erecta ecotype.

In an attempt to identify new genes regulating the ABA response in germination, we have initiated a screen for ABA-insensitive mutants in plants expressing random GFP:DNA fusions (Columbia ecotype). These fusions were generated from genomic DNA as well as mRNA isolated from etiolated seedlings and callus tissue. This approach can potentially identify new dominant mutations that may help in understanding the role of ABA in germination. We present the characterization of mutants identified in this screen.

323 The allene oxide cyclase family of *Arabidopsis thaliana*

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Jasmonates and octadecanoids are signals in plant stress responses and development. An essential step in jasmonate biosynthesis is catalyzed by the allene oxide cyclase (AOC), where the correct stereochemistry of the precursor 12-oxyphytodienoic acid is established. In tomato a single copy gene codes for an AOC which occurs specifically in all vascular bundles and ovules of young flower buds and attributes to amplification in wound signalling[1].

In *Arabidopsis thaliana* the AOC is encoded by four genes which are transiently and differentially up-regulated upon wounding both locally and systemically[2]. In contrast, AOC protein appeared at constitutively high basal level. Immunocytochemical analyses revealed abundant occurrence of the first three enzymes in JA biosynthesis, the lipoxygenase, the allene oxide synthase and the AOC. Using the JA-deficient mutant *opr3*, a positive feed back regulation in JA biosynthesis is suggested for leaf development. JA biosynthesis is only activated upon substrate generation. Immunocytochemical analyses of cross-sections and longitudinal sections of flowers revealed occurrence of AOC protein in most tissues and cell types except pollen. AOC protein appeared abundantly in all vascular bundles, ovaries and stigma cells. Functional analysis of the four AOCs by promoter::*uidA* lines revealed non-redundant activity in flower development and the wound response of leaves. Transgenic lines were generated with a RNA*i* construct containing inverted repeats of *AOC3* exon2 which shows the highest homology to all four *AOCs*. Several lines exhibited deficiency in AOC and JA content. Consequences of this deficiency in terms of altered phenotype and gene expression will be presented.

1. Stenzel, I., Hause, B., Maucher, H. Pitzschke, A., Miersch, O., Ziegler, J., Ryan, C.A. and Wasternack, C. Allene oxide cyclase dependence of the wound response and vascular bundle-specific generation of jasmonates in tomato - amplification in wound signaling. *Plant J*. 33, 577-589 (2003)
2. Stenzel, I., Hause, B., Miersch, O., Kurz, T., Maucher, H., Weichert, H., Ziegler, J., Feussner, I. and Wasternack, C. Jasmonate biosynthesis and the allene oxide cyclase family of Arabidopsis thaliana. *Plant Mol. Biol*. 51, 895-911 (2003)

324 Auxin response is mediated by a family of related SCF complexes

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Plant responses to the phytohormone auxin require the regulated degradation of Aux/IAA proteins. This degradation requires the ubiquitin protein ligase SCF^{TIR1} consisting of CUL1, ASK1/ASK2, RBX1 and the F-box protein TIR1. However, the relatively mild and essentially root specific phenotypes of *tir1* null mutants raise the possibility that other F-box proteins may also function in auxin response. Here we report the characterization of a family of F-box proteins related to TIR1, designated as LRF (Leucine-rich Repeat F-box) proteins. To investigate whether LRF proteins act as SCF components, we expressed myc tagged version of each LRF in Arabidopsis. Co-immunoprecipitation of LRF-myc with the SCF subunits, ASK1 and CUL1 confirmed that the LRFs are found in SCF complexes. To determine if the Aux/IAA proteins are targets for SCF^{LRFs}, we carried out pull-down assays using GST-IAA7 or GST-IAA17. We found that LRF1-myc, LRF2-myc and LRF3-myc all interacted with GST-IAA proteins in an auxin dependant manner suggesting that Aux/IAA proteins are substrates for SCF^{LRFs}. T-DNA insertion mutants were isolated for LRF1, LRF2 and LRF3. Mutations in LRF1, the closest relative to TIR1 did not confer a phenotype. In contrast, *lrf2* mutants displayed a strong auxin resistant root phenotype while *lrf3* mutants had a weaker phenotype in roots. To study the cumulative effect of LRFs in auxin response, we created a series of double and triple mutant lines between the members of the family including TIR1. Sequential knockout of each member of the family generated increasingly severe aerial and root phenotypes. Remarkably, *lrf2 lrf3 tir1* triple mutants exhibit a severe seedling lethal phenotype similar to the monopteros and bodenlos mutants. These results suggest that auxin response is mediated by a family of related SCFs throughout plant development.

325 Dual regulation of an Arabidopsis cysteine protease by sugar starvation and the serine/threonine protein phosphatase inhibitor calyculin A.

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Sugar starvation is a common event in the life of a plant, and sugar starvation is a signal that has widespread regulatory influences on metabolism and gene expression, which in turn impact on photosynthesis and developmental processes such as flowering and senescence. However, the mechanisms by which plants sense sugar starvation and transmit starvation signals are not well understood. Here, we have purified and identified a protease that was present in extracts of sugar starved (but not sugar fed) cells. Two signalling pathways have been identified that trigger the expression of the active protease in Arabidopsis suspension cells, and our findings indicate cross talk between sugar starvation and pathogen-defence signalling. First, protease protein and activity were induced in cells starved of metabolised sugars. Second, the protein phosphatase inhibitor calyculin A triggered a faster and stronger expression of the protease than did starvation. Using inhibitors of protein kinases to dissect the regulation of protease expression, we found that selective inhibitors of the MAP kinase cascade blocked the response to calyculin A but not to starvation, while H89 (a potent PKA inhibitor) blocked the appearance of protease activity in both starved and calyculin A-treated cells. It has been shown that specific MAP kinases become activated during plant defence responses, and several defence responses of plants are known to be induced by protein phosphatase inhibitors. Thus, we are testing the hypothesis that the calyculin A-induced pathway to protease induction is mimicking a plant defence response pathway that is distinct from, but shares elements of, the sugar starvation-induced signalling pathway.

326 The Role of Auxin Response Factors in Plant Development

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The hormone auxin influences many aspects of plant growth and development. Auxin signaling is controlled by a number of mechanisms including transcriptional regulation, which is mediated in part by the auxin response factor (ARF) family of transcription factors. The Arabidopsis genome contains 22 ARF genes that have the ability to either activate or repress the expression of auxin-responsive genes. ARF proteins are typically comprised of an N-terminal DNA-binding domain that can bind to the auxin response element TGTCTC, a middle region that can activate transcription, and a C-terminal domain bearing homology to domains III and IV of Aux/IAA proteins. The heterodimerization of ARFs with Aux/IAA proteins allows for regulation of ARF activity. In an attempt to define the roles of individual ARF genes in plant development, we have isolated plants from T-DNA insertion collections and have used RNAi technology to create transgenic lines with reduced ARF gene expression. In addition, select double mutants have been made from phylogenetically related single arf mutants, which have revealed some redundancy in ARF gene function. The phenotypes of these mutants will be discussed.

327 CSN Interacts with SCF^{COI1} and Modulates Jasmonate Responses

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The COP9 signalosome (CSN) is an evolutionarily conserved, nucleus-enriched multiprotein complex. CSN plays roles in photomorphogenesis, auxin response, and floral organ formation, possibly via the regulation of ubiquitin-proteasome mediated protein degradation. *COI1* encodes an F-box protein, which is a subunit of SCF^{COI1} E3 ubiquitin ligase, and is required for jasmonate (JA) responses. Here, we demonstrate using coimmunoprecipitation and gel-filtration analyses that endogenous as well as epitope-tagged COI1 forms SCF^{COI1} and associates directly with CSN in vivo. Like the *coi1-1* mutant, CSN reduction-of-function plants exhibited a JA-insensitive root elongation phenotype and an absence of JA-induced-specific gene expression. Genome expression profile analyses indicated that JA-triggered genome expression is critically dependent on COI1 dosage. More importantly, most of the *COI1*-dependent JA-responsive genes also required CSN function, and CSN abundance was shown to be important for JA responses. Furthermore, we showed that both *COI1* and CSN are essential for modulating expression of genes in most cellular pathways responsive to JA. Thus, CSN and SCF^{COI1} work together to control genome expression and promote JA responses.

328 Auxin promotes Arabidopsis root growth by modulating gibberellin response

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The growth of plant organs is influenced by a stream of the phytohormone auxin that flows from the shoot apex to the tip of the root. However, until now, it was not known how auxin regulates the cell proliferation and enlargement that characterizes organ growth. Here we show that auxin controls the growth of roots by modulating cellular responses to the phytohormone gibberellin. GA promotes the growth of plants by opposing the effects of nuclear DELLA protein growth repressors. GA opposes the action of several DELLA proteins by destabilising them via proteasome-mediated protein degradation pathway, reducing both the level of detectable DELLA proteins and their growth-restraining effects. Here,we show that shoot apex-derived auxin is necessary for GA-mediated control of root growth. Furthermore, we show that attenuation of auxin transport or signalling delays the GA-induced disappearance of DELLA protein from root cell nuclei. Our observations indicate that the shoot apex exerts long-distance control on the growth of plant organs via the effect of auxin on GA-mediated DELLA protein destabilisation.

329 Analysis of natural variation of auxin regulated traits and QTL mapping.

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The plant hormone auxin plays a major role in many aspects of plant growth and development. Quantitative genetics might offer a suitable approach to better understand how different traits are controlled by auxin at gene and whole genome levels. To address whether natural variation in Arabidopsis might be a source of phenotypic and genetic information, a total of 120 wild accessions were tested at the seedling stage for traits regulated by auxin. First, hypocotyl length at 21 and 29C was determined. A smaller number of accessions consisting of those displaying extreme and opposite variations in hypocotyl growth at 29C were also tested for root growth rate, lateral root formation and root growth inhibition in presence of auxin. An extensive degree of phenotypic variation was found for all the traits analyzed. This variation seems to develop from different genetic backgrounds according to genetic distances previously calculated in our lab for the accessions used in this work. A positive correlation was found between hypocotyl lengths at 21 and 29C suggesting that similar QTLs might be involved in the control of these traits. The same was true among the root traits analyzed. No correlation was found between hypocotyl length at any temperature and root traits. Next the QTL mapping of hypocotyls length at 21 and 29C was performed using a new set of RILs derived from a Ler x No-0 cross. A total of 97 lines mapped for 31 microsatellites were assayed for hypocotyl length. A QTL interval mapping analysis reveled a major QTL common to both conditions linked to marker PSL5 in chromosome 2 and possibly related to light control. Another major QTL was exclusively detected at high temperature, linked to marker JV57/58 in chromosome 5. It has been described that auxin plays a major role during the elongation of hypocotyls at high temperature suggesting that the QTL found in chromosome 5 might be auxin related. In fact, we found several candidate genes involved in auxin signal transduction in this specific QTL region. The QTL mapping of root traits is underway.

330 Catabolism of Abscisic Acid by Cytochrome P450-mediated Hydroxylations

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The phytohormone (+)-abscisic acid (ABA) plays a crucial regulatory role in plant growth and development and in responses to environmental stress. The concentration of ABA *in planta* is the result of a highly regulated balance between biosynthesis, catabolism and transport. The framework of the ABA biosynthetic pathway is known and the genes involved have been identified. However, far less is known about ABA catabolism. The first step of the principal catabolic pathway involves the oxidation of ABA to 8'-hydroxy-ABA followed by cyclization to phaseic acid. An apparently minor alternative pathway involves 7'-hydroxylation. Previously, we confirmed that the first enzyme in the main pathway, ABA 8'-hydroxylase (A8H), is a cytochrome P450 monooxygenase (Krochko *et al*, 1998; Plant Physiol. 118:849-860). We have now identified several cytochrome P450 genes from *Arabidopsis* involved in oxidative ABA catabolism and we will report biochemical and physiological data for one of these gene products, A8H-1. RT-PCR analysis revealed that A8H-1 is expressed at low levels in a number of tissues. *Arabidopsis* plants overexpressing A8H-1 exhibited subtle alterations in phenotype and little overall change in the level of endogenous ABA and related metabolites. However, in these transgenic plants, feeding studies using labeled ABA revealed increased ABA 8'-hydroxylation, and sometimes 7'-hydroxylation. Furthermore, transgenic seed germinated more rapidly in the presence of ABA than wild-type seed. In *Arabidopsis*, the fact that there appear to be several genes with overlapping catabolic functions is likely why phenotypic screens of mutagenized populations have so far failed to recover an ABA catabolism mutant.

331 Molecular characterization of *PIN* genes encoding components of auxin efflux carriers

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Auxins regulate a wide array of growth and developmental processes including tropic responses to light and gravity, elaboration of lateral organs such as flowers and lateral roots, differentiation of vasculature, establishment of apical dorminance and patterning during embryogenesis and vegetative growth. Unlike other plant hormones, auxins are unilaterally transported in plants from sites of synthesis at shoot apex and young leaves to sites of action in a process termed as polar auxin transport. Polar auxin transport has been implicated in auxin-regulated developmental processes. Furthermore, recent experimental evidence suggests that polar auxin transport is closely linked to auxin responses. Elucidating mechanisms of how auxin is transported into and out of transporting cells not only is important for our understanding of how auxin transport is regulated, but also may shed light on upstream events of auxin signal transduction. We are using a functional genomics approach to characterize several T-DNA insertional mutants in *PIN* genes that encode components of the auxin efflux carriers. In addition, we have performed immunolocalization experiments with specific polyclonal antibodies raised against purified truncated PIN proteins to localize PIN proteins. We are also using an auxin-responsive reporter (*DR5:GUS*) to monitor auxin distribution *in planta* in both wild type and *pin* mutants. With these approaches, we are testing the involvement of actin cytoskeleton, protein phosphorylation and NPA-binding proteins in PIN protein function.

332 Over-expression of CSN5 JAMM mutations in Arabidopsis cause a pleiotropic dominant negative phenotype

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The COP9 signalosome (CSN) is an evolutionary conserved multi-protein complex that was originally identified in Arabidopsis as a negative regulator of photomorphogenic seedling development. The COP9 complex has been linked to the integration of signaling pathways even if its exact role is still obscure. Both in plants and yeast, the CSN regulates the SCF E3 ubiquitin-ligase complex by mediating the removal of RUB from the Cullin subunit. One subunit of the COP9 complex, CSN5/Jab1, contains a MPN metalloprotease motif (JAMM) that is required for CSN?s RUB isopeptidase activity in yeast. In Arabidopsis CSN5 is encoded by two evolutionary conserved genes (CSN5A and 5B). Here we report the identification of a knockout insertion line in CSN5B. Homozygous csn5B mutants display a wild-type phenotype and accumulate Rubbylated and un-Rubbylated Cul1. To further investigate the role of CSN5 in Arabidopsis we generate single point mutations in several key residues within or adjacent to the JAMM domain, both in CSN5A and CSN5B proteins. The csn5b homozygous plants over-expressing MYC-tagged CSN5A or CSN5B wild-type proteins display wild-type phenotype. By contrast, ectopic expression of MYC-CSN5A with different point-mutation in the JAMM domain (into csn5b mutant background) result in a severe dwarf and branching phenotype, suggesting a dominant negative interference with the activities of the endogenous CSN5A. All the mutated proteins co-fractionate with other CSN subunits, indicating that the point mutations do not interfere with the ability of the proteins to assemble into the CSN complex. The phenotype displayed by these lines is characterized by curly shaped leaves, a strong increase in the number of secondary inflorescences, and a drastic reduction in plant size and internode length, with several traits resembling the lost of apical dominance of auxin-resistant mutants. Moreover these lines present abnormal siliques, both in length and shape and a partial sterility phenotype. Accordingly, these plants also display an alteration in the pattern of AtCul1, with an increase accumulation of the RUB-modified form with respect to csn5b mutant and wild type plants.

333 Over-Expression And Antisense-Suppression Of The PERK1 Receptor Kinase in *Arabidopsis* Leads To Changes In Growth And Seed Production

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The PERK receptor kinase family is characterized by an extracellular domain that is proline-rich and extensin-like followed by a transmembrane domain and kinase domain. The original member, PERK1, was isolated from *Brassica napus*, and 14 PERK1-related members were subsequently identified in the *Arabidopsis* genome. One *At*PERK member was readily identifiable as the orthologue of *Bn*PERK1 based on high similarities in sequence identity, domain organization, and expression patterns. Overexpression and antisense suppression experiments were performed using the *Bn*PERK1 cDNA under the control of the 35S CaMV promoter and introduced into *Arabidopsis thaliana* Col-0. In the case of the antisense suppression, the *Bn*PERK1 cDNA shared sufficient sequence similarity to potentially suppress all 14 *At*PERK members. In both sets of transgenic *Arabidopsis*, several heritable changes in growth and development were observed. The overexpressing PERK1 *Arabidopsis* plants showed increases in height, secondary branching, root growth and seed production as well as loss of seed dormancy when compared to the wild-type *Arabidopsis* Col-0 plants. The PERK1 antisense suppressing transgenic plants showed various defects ranging from lethal phenotype with seedlings completely lacking roots to a less severe phenotype with loss of apical dominance and partial sterility compared to the wild-type *Arabidopsis* Col-0 plants. We are currently screening for T-DNA insertions in individual *At*PERK members to examine the individual contributions of these genes to the observed phenotypes.

334 A cell surface receptor mediates extracellular calcium sensing in Arabidopsis guard cells

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The role of extracellular calcium (Ca2+o), serving as calcium pool for the changes of cytosolic calcium ([Ca2+]i), a very important second messenger, in response to environmental and developmental signals is now well known. But less understood is whether Ca2+o can also function as a primary messenger in plants. The effects of Ca2+o on guard cells in promoting stomatal closure via triggering [Ca2+]i increases and oscillations provide a suitable system for dissecting Ca2+o signal transduction pathway in plants. Using cameleon-based Ca2+-imaging techniques we investigated the molecular mechanism of Ca2+o-induced [Ca2+]i increases (CICI) and found that Ca2+o is perceived by a receptor that triggers [Ca2+]i increases in Arabidopsis guard cells. Then we used an expression cloning strategy in mammalian cells to isolate an Arabidopsis cDNA clone encoding a Ca2+o -sensing receptor (CAS). CAS is expressed in guard cells, and localized to the plasma membrane. Elimination of CAS expression disrupts Ca2+o -induced [Ca2+]i increase in guard cells and stomatal closure. These data indicate that CAS may be a primary transducer of extracellular calcium in Arabidopsis guard cells.

335 Structure-function studies of NPH3, a phototropin 1-interacting protein

Johanna Harris, Renee Harper, Andrei Motchoulski and Emmanuel Liscum

Phototropism is an excellent system for studying how plants perceive light cues and use this input to optimize growth in a constantly changing environment. In recent years knowledge of how plants identify and respond to directional light has increased dramatically, due in part to studies of the *nph* (non-phototropic hypocotyl) mutants of Arabidopsis. The *nph3* mutant was isolated in a screen for seedlings un-responsive to unilateral blue light (BL). The *NPH3* gene has been found to encode a novel plant-specific protein that interacts with phot1, the primary photoreceptor for low fluence rate BL-induced phototropism. While little is known about the biochemical function of NPH3 it is hypothesized to function as a scaffold protein that links early-signaling events of the phototropic pathway. In attempts to elucidate NPH3 function and associated events in the phototropic signaling pathway, we are using multiple approaches to identify structural motifs within NPH3 that are critical for proper signaling. One approach has been to assess the ability of various mutant versions of NPH3 (e.g., TN5-generated 19-amino acid insertions or domain swaps with other member of the NPH3/RPT2 family) to complement the aphototropic phenotype of a *nph3*-null mutant. A second approach has been the analysis of a series of TILLING (targeting induced local lesions in genomes) alleles of *nph3* that result in single amino acid substitutions within various regions of the protein mutations in NPH3. These approaches will provide information on multiple levels, including structure-function information and the possibility to do genetic suppressor screens to identify other NPH3-interacting proteins. Relative to this latter aspect, we have already targeted one TILLING allele for second-site mutation screening. Results from the each of the aforementioned studies will be presented.

336 Molecular characterization of the intracellular target protein for the receptor kinase IRK expressed in inflorescence meristem in Arabidopsis

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Families of receptor-like kinase (RLKs) play important roles in plant development. Extracellular information is conceivably transduced by ligand-dependent RLK dimerization that causes self-phosphorylation of kinase domain and subsequent phosphorylation of interacting target molecules by the kinase domain. We have isolated a novel receptor-like kinase (IRK) by differential screening of genes expressed in low level in the inflorescence meristem from equalized cDNA library of *Arabidopsis thaliana*. The IRK contains LRR type receptor domain, transmembrane domain and cytoplasmic serine/threonine kinase domain. Expression studies revealed that *IRK* expresses at inflorescence meristem, floral organs and root tip (Kanamoto et al., 2002). To identify cytoplasmic target of IRK, we performed a yeast two-hybrid screening with the IRK kinase domain as bait. The candidate target protein of IRK (IRKT) isolated by this screening was a novel protein, although it showed weak homology with kinesin in the central region and with cadherin repeat in the C-terminal region. IRKT was specifically interacted to the IRK kinase domain by in vitro assays. The simultaneous expression of *IRKT* and *IRK* in shoot apecies supported that interaction occurs in plants. In order to clarify the function of IRKT, T-DNA tag line and over expressing line were analyzed, but they showed no phenotype. Currently genetic analysis of IRKT is in progress, taken genetic redundancy into account.

H. Kanamoto, J. Hattan, M. Takemura, A. Yokota, and T. Kohchi, 2002. XIII International Conference on ARABIDOPSIS RESEARCH, 4-53

337 The CUL1 protein is required for auxin signaling in Arabidopsis

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The AXR6 gene is required for auxin signaling in the Arabidopsis embryo and during postembryonic development. One of the effects of auxin is to stimulate degradation of the Aux/IAA auxin response proteins through the action of the ubiquitin protein ligase E3 SCFTIR1. Here we show that AXR6 encodes the SCF-subunit CUL1. The axr6 mutations affect the ability of mutant CUL1 to assemble into stable SCF complexes resulting in the stabilization of the SCFTIR1 substrate AXR2/IAA7 and reduced auxin response on transcriptional level. These results strongly support the developing model of auxin signal transduction, in which the ubiquitin pathway plays a central role.

338 HDZip Proteins Are Potential Regulators of Abscisic Acid Responsiveness in *Arabidopsis thaliana*

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The Arabidopsis homeodomain-leucine zipper (HDZip) transcription factors, ATHB5, -6 and-16, are closely related and belong to HDZip I, one of the four major HDZip families. *In vitro*, ATHB5, -6 and -16 bind to pseudopalindromic DNA sequences; CAATNATTG, as protein dimers. In transient expression assays on Arabidopsis leaves ATHB5, -6, and -16 can activate transcription of reporter gene constructs with upstream CAAT(A/T)ATTG binding sites, thus implying that ATHB5, -6 and -16 function as transcriptional activators *in vivo*. *ATHB5, -6* and *16* are all regulated by abscisic acid (ABA) at the transcriptional level, but in different manners. Plants carrying a loss-of-function mutation in *ATHB5* or *ATHB6* does not show phenotypic alterations, but plants overexpressing *ATHB5, -6* or *-16* show a changed sensitivity to ABA on seed germination assays and on root growth assays. This indicates that ATHB5, -6 and -16 act as regulators of different aspects of the growth response to ABA. Phenotypes of plants having an altered level of *ATHB16* expression suggest that ATHB16 is also a component in the light sensing mechanism of the plant.

339 Mutational Studies of phyA Signaling Intermediates and Their Roles in the Modulation of phot1-Dependent Phototropism

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Plants respond adaptively to environmental cues in order to maximize their growth potential. For example, plants growing in poor light environments utilize a variety of mechanisms, such as shade-avoidance responses and phototropism, to alter their growth characters to maximize light interception necessary to drive photosynthesis and development. While phototropism is generally induced via BL signals perceived by the phototropin (phot) receptors in higher plants, the predominantly red/far-red-absorbing phytochrome (phy) receptors mediate shade-avoidance responses and can modulate phototropin-dependent phototropism. As part of our ongoing attempts to understand the molecular basis of phyA modulation of phot1-dependent phototropism we are analyzing the phototropic response of seedlings singly, doubly, and multiply mutant for each of the four members of the *PKS (PHYTOCHROME KINASE SUBSTRATE)* gene family in Arabidopsis. The founding member of this family, PKS1, was found in a two-hybrid screen for proteins that interact with the C-termini of both phyA and phyB, and was subsequently shown to be phosphorylated *in vitro* by phyA. It has been proposed that PKS1 sequesters phytochromes in the cytoplasm to negatively regulate their response to light, thus loss-of-function mutations are predicted to be phototropically hypersensitive. In a parallel but distinct approach we have identified second-site mutations in the *nph4/arf7*-null mutant background that fail to exhibit the phyA-dependent component of phototropism and are now using map-based cloning techniques to isolate one of these loci, *MAP3* (mutant designation, *map3* for modifier of *nph4/arf7* phenotypes), which appears to encode a protein functioning as a positive regulator of this phyA pathway uniquely.

340 Arabidopsis *ARGOS*, a novel auxin-inducible gene involved in lateral organ size control

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During plant development, the organ size is affected by various developmental signals, but the molecular mechanisms governing these size signals controlling are largely unknown. Here we show that *ARGOS*, a novel Arabidopsis gene that is highly induced by auxin, is involved in organ size control. Transgenic plants expressing sense or antisense *ARGOS* cDNA exhibit enlarged or reduced size of aerial organs, respectively.The chang in organ size is mainly due to changes in cell number and the duration of organ growth. Ectopic expression of *ARGOS* prolongs the expression of *ANT* and *CycD3;1*, as well as the neoplastic activity of leaf cells, suggesting that ARGOS plays a role through the maintenance of cell meristematic competence. Furthermore, the induction of *ARGOS* by auxin is attenuated or abolished in *axr1* mutants, and overexpression of *ARGOS* partially restores the organ development of *axr1* mutant. These results suggest that ARGOS transduces auxin signals downstream of AXR1 to regulate cell proliferation and organ growth during organogenesis.

341 Molecular genetic analysis of signal transduction related genes of BR

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A specific inhibitor of the plant hormone is a very useful tool to study its function. Brassinazole(BRZ) is a triazole compound that specifically blocks brassinoride(BR) biosynthesis by inhibiting cytochrome P450 steroid C-22 hydroxylase encoded by the DWF4 gene. To expand our knowledge of the molecular mechanisms of plant steroid signaling, we performed a genetic screen in which 170 pools of 100 activation tagged Columbia lines in the dark on medium containing brz. Mutants insensitive to a BR biosynthetic inhibitor, brassinazole were shown to have long hypocotyls than the wild type background. We Identified 6 mutants that also have long petioles and recovered T-DNA flanking sequences from 4 activation tagged brassinazole insensitive(*abz*) mutants. We have cloned the abz mutants using adopter ligation PCR walking. *abz126* is a knock out of *Gigantea(GI)* gene by T-DNA insertion that delayed flowering under long days and displayed longer hypocotyls on medium containing brassinazole, and also *gi1* and *gi2* mutants which have a deletion of GI displayed insensitive brz treatment. *abz126* showed insensitive response in pacrobutrazole which blocks gibberellin biosynthetic pathway but showed altered response in BAP same as Columbia. These results suggest that brassinosteroids and gibberellins signal transduction negatively act for hypocotyls elongation through GI. Another approach to elucidate brassinosteroid signalling would be to screen for bri1 activation-tagged suppressor mutants. Genetic screens designed for gain-of-function mutations are particularly useful in uncovering genes that cause no phenotypes when inactivated, either due to redundancy or lethality. We identified 8 bri1 suppressor mutants on medium containing brz from 7500 T1 lines. The isolation and investigation of the bri1 suppressor will greatly contribute to identify other signaling components in the pathway and understand regulation and action of brassinosteroid signal pathway in plants.

342 A Seven-transmembrane RGS Protein Modulates Cell Proliferation in Arabidopsis

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Heterotrimeric G-proteins are activated by seven-transmembrane (7TM) cell-surface receptors that induce the G-protein alpha (Gα) subunit to exchange GDP for GTP. Regulators of G-protein signaling (RGS) proteins accelerate the deactivation of G proteins to reduce GPCR signaling. Here we identify the first RGS protein in Arabidopsis, designated AtRGS1. This protein is predicted to have the structural features of a 7TM receptor as well as a C-terminal RGS-box. The RGS-box of AtRGS1 binds the Arabidopsis Gα subunit AtGPA1, accelerates its GTPase activity, and complements the pheromone supersensitivity phenotype of the yeast RGS mutant, sst2Δ. Both AtRGS1-GFP and AtGPA1-GFP localize to the plasma membrane. In dividing cells, AtRGS1-GFP and AtGPA1-CFP accumulate at the nascent cell plate. Increase in the activity of AtGPA1, either by expression of GTPase-deficient AtGPA1 or by loss of AtRGS1 expression, results in increased cell elongation in hypocotyls grown in darkness and increased cell production in roots grown in light. Collectively, these findings suggest that AtRGS1 is a critical modulator of plant cell proliferation.

343 Identification and Characterisation of *AXR-3*-interacting proteins.

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Aux/IAAs make up a large family of transcription factors that interact with Auxin Response Factors (ARFs) to bring about the expression or repression of auxin inducible genes. AXR3 was one of the first two Aux/IAA proteins to be identified as a target of the SCF^{TIR1} ubiquitin protein ligase, central to auxin signalling. Of all the Aux/IAAs, AXR3 is one of the most studied. Present interest is in the identification and characterisation of proteins that interact directly or indirectly with AXR3, which may reveal vital information about auxin signalling.

The *PAX1* gene was identified because mutation at *PAX1* results in partial suppression of the *axr3-1* gain-of-function phenotype. The *pax1-1* mutation has already been found to interact genetically with other Aux/IAAs and reduce GUS expression from an AXR3promoter:GUS construct, suggesting that PAX1 may upregulate transcription of *AXR3*. Insight into whether the *pax1-1* mutation suppresses the *axr3-1* phenotype by altering the transcription and/or translation of the AXR3 gene, or the stability of the mutant protein is being gleaned from analysis of three translational GUS fusions. The cloning of this gene by map based methods is underway.

A type 1 phosphoprotein serine/threonine phosphatase (PP1c) isoform 1 protein, known as TOPP1, interacts with AXR3 in the yeast-2-hybrid system. Pull-down assays will be performed to obtain confirmation of this interaction in bacteria and subsequently *in planta*. The expression pattern of TOPP1 will be revealed using a TOPP1promoter:GUS construct. The effect of a mutation in *TOPP1* is being determined *in planta* via analysis of a T-DNA insertion line from the Versailles collection and two lines obtained though the *Arabidopsis* TILLING Project.

344 Characterization of Abscisic Acid 8'-Hydroxylase Activity from Corn Suspension Cells

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Abscisic acid (ABA) is a key phytohormone influencing plant growth and development as well as adaptive responses to environmental stresses. The effective concentrations of abscisic acid in plant tissues are determined by opposing forces of import/export and synthesis/degradation. The enzyme ABA 8'-hydroxylase catalyzes the first step in the oxidative catabolism of (+)-ABA and is considered the pivotal enzyme controlling the degradation of ABA in plants. Corn suspension cells (Black Mexican Sweet) develop an increased capacity to metabolize ABA following the addition of 10 to 200 micromolar ABA to the media (Cutler et al 1997, Krochko et al 1998). Utilizing this system we have developed an [I]in vitro[/I] assay for this enzyme using microsomal extracts from (+)-ABA-induced corn suspension cells. ABA 8'-hydroxylase is an integral membrane protein, requires oxygen and NADPH for activity, is inhibited by tetcyclacis and carbon monoxide, and shows blue-light reversibility of the carbon monoxide inhibition. These features are consistent with the classification of this enzyme as a cytochrome P450 monooxygenase. Using a subtractive hybridization protocol (Clontech PCR-Select cDNA Subtraction Kit) we have isolated a 333 bp cDNA fragment of a cytochrome P450 enzyme from an ABA-induced subtracted cDNA library. Functional characterization of this P450 enzyme using a full-length maize cDNA has confirmed ABA hydroxylase activity.

Krochko JE et al (1998) (+)-Abscisic acid 8'-hydroxylase is a cytochrome P450 monooxygenase. Plant Physiol 118: 849-860. Cutler AJ et al (1997) Induction of (+)-abscisic acid 8'-hydroxylase by (+)-abscisic acid in cultured maize cells. J Exp Bot 48: 1787-1795.

345 Transcriptional Regulation by the Auxin Response Factor MONOPTEROS

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The Arabidopsis *MONOPTEROS* (*MP*) gene plays a central role in embryo axis and vascular strand formation. *MP* encodes a transcription factor of the 'Auxin Response Factor' (ARF) family, whose members regulate the expression of auxin-induced genes by binding to conserved promoter 'Auxin Response Elements' (AuxREs). Several lines of evidence have implicated auxin in cell pattern formation. MP could therefore relay auxin signals in embryo axis patterning and vascular differentiation. Its target genes, however, are unknown.

We have determined the expression profiles of numerous genes involved in vascular development and auxin signaling in backgrounds of varying MP activity. This has revealed many candidate MP transcriptional targets. To establish which of these MP-dependent genes are direct downstream targets, a *mp* mutant line has been generated containing a posttranslationally inducible *MP* transgene (employing the hormone binding domain of the glucocorticoid receptor). This *MP:GR* transgene successfully and reversibly rescues the *mp* mutant, providing a suitable tool for elucidating direct targets. The most promising target revealed from this inducible system, the HD-ZIP III gene *ATHB-8*, has clear putative AuxREs in noncoding regulatory regions, further suggesting dependence on MP for its expression. This *MP:GR* line is currently being studied by microarray analysis to reveal potential downstream MP targets on a genome-wide scale.

346 NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in Arabidopsis

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Guard cells have been developed for dissecting early signal transduction mechanisms. Despite large efforts relatively few signal transduction components have been identified from recessive ABA insensitive disruption mutants known to function during early ABA signal transduction upsteam of transcription. The limited number of genetically identified positive ABA transducers is most likely due to redundancy in genes encoding ABA signaling components. To overcome this limitation and to dissect redundant signal transduction proteins, we have developed an alternative "single cell-type genomics" approach.

Reactive oxygen species (ROS) have been proposed to function as second messengers in ABA signaling in guard cells (Pei *et al*., 2000, *Nature* 406: 731-734). However, the question whether ROS production is rate-limiting for ABA signal transduction *in vivo* has not yet been addressed and the molecular mechanisms mediating ROS production during ABA signaling remain unknown. Two partially redundant *Arabidopsis* guard cell-expressed NADPH oxidase catalytic subunit genes, *AtrbohD* and *AtrbohF*, were identified in which gene disruption impairs ABA signaling. *atrbohD/F* double mutation impairs ABA-induced stomatal closing, ABA promotion of ROS production, ABA-induced cytosolic Ca^{2+} increases and ABA-activation of plasma membrane Ca^{2+}-permeable channels in guard cells. Exogenous H_2O_2 rescues both Ca^{2+} channel activation and stomatal closing in *atrbohD/F*. These data provide direct molecular genetic and cell biological evidence that ROS function as second messengers in ABA signaling and that the AtrbohD and AtrbohF NADPH oxidases function in guard cell ABA signal transduction. Further new data on analyzing roles of ROS generation in guard cell signaling will be presented.

347 Arabidopsis constitutive photomorphogenic mutant brl1 shows altered brassinosteroid and sugar response

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The regulation of plant growth and development by environmental signals requires the action of some endogenous factors, including phytohormones. During a screen for photomorphogenic mutants, we isolated a mutant which was defective in brassinosteroid responses as well and thus designated *brl1* (*br*assinosteroid and *l*ight 1). The mutant *brl1* shows short hypocotyls, expanded cotyledons, and derepression of light regulated genes in young seedlings and leaf differentiation and silique formation on prolonged growth in dark. In light, the mutant brl1 is dwarf and develops a short root, compact rosette, less trichomes, and exhibits delayed bolting. The short hypocotyl phenotype in dark and short root phenotype in light was rescued by exogenous brassinosteroid application. The mutant also shows reduced activity of the BR inducible *TCH4* and auxin inducible *SAUR* promoters fused with GUS gene. In addition, the mutant is hypersensitive to metabolizable sugars, which can be rescued with BR, suggesting that the primary lesion is in the BR response pathway. The mutant *brl1* is non-allellic to brassinosteroid biosynthetic mutants, *cpd* and *det2*, which also show photomorphogenesis in dark. Using a genome-wide AFLP mapping strategy, the mutant has been mapped to a 1.4 Mb region of chromosome 5. Further analysis revealed that no other BR-related mutant has yet been identified in this region, suggesting that the mutant *brl1* defines a novel locus involved in BR biosynthesis.

348 Characterization of Overexpression of Gibberellin 2-Oxidases from Spinach

Dong Ju Lee and Jan A.D. Zeevaart

Michigan State University

Characterization of Overexpression of Gibberellin 2-Oxidases from Spinach in *Arabidopsis* and Tobacco Dong Ju Lee and Jan A.D. Zeevaart MSU-DOE Plant Research Lab., Michigan State University, E. Lansing MI 48824 Gibberellin 2-oxidases are a family of dioxygenases that inactivate gibberellins (GAs). The objective of this study is to understand the function of *SoGA2ox1* and *SoGA2ox2* genes from spinach through overexpression lines in *Arabidopsis* and tobacco. In previous research, SoGA2ox1 and SoGA2ox2 proteins converted C_{19}-GAs (GA_{20} and GA_1) to the inactive products GA_{29} and GA_8, respectively. To determine whether the overexpression would cause a GA-deficient phenotype in different species, the *35S::SoGA2ox1* and the *35S::SoGA2ox2* cDNA fusion constructs were transformed into *Arabidopsis* and tobacco (*Nicotiana sylvestris*), respectively. These overexpression lines have typical GA-deficient phenotypes, such as low rate of seed germination, short hypocotyl, small rosette size, small and dark-green leaves, short stem, small root system, and late flowering. Leaves are short and curled. Internode length is also decreased. Transgenic lines are defective in floral development. Flowers of transgenic *GA2ox2* lines are smaller (60%) than those of wild type plants and their stamens and ovaries are not well developed in tobacco. These phenotypes can be partially overcome by treatment with GA_3. The more severe dwarfism, the less effective the treatment with GA_3 is. Most seeds (90%) of WT and transgenic *GA2ox1* lines have germinated 3 days after sowing on agar medium. However, transgenic *GA2ox2* lines have a lower germination rate (50%) than those of WT plants and transgenic *GA2ox1* lines in *Arabidopsis*. These results indicate that the SoGA2ox1 and SoGA2ox2 proteins are functional in *Arabidopsis* and tobacco plants, and that some of their functions are different, even though they have the same enzymatic activity. (Supported by USDA grant no. 2002-35304-12693 and DOE grant no. DE-FG02-91ER20021).

349 Characterization of Overexpression of Gibberellin 2-Oxidases from Spinach

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Characterization of Overexpression of Gibberellin 2-Oxidases from Spinach in *Arabidopsis* and Tobacco Dong Ju Lee and Jan A.D. Zeevaart MSU-DOE Plant Research Lab., Michigan State University, E. Lansing MI 48824 Gibberellin 2-oxidases are a family of dioxygenases that inactivate gibberellins (GAs). The objective of this study is to understand the function of *SoGA2ox1* and *SoGA2ox2* genes from spinach through overexpression lines in *Arabidopsis* and tobacco. In previous research, SoGA2ox1 and SoGA2ox2 proteins converted C_{19}-GAs (GA_{20} and GA_1) to the inactive products GA_{29} and GA_8, respectively. To determine whether the overexpression would cause a GA-deficient phenotype in different species, the *35S::SoGA2ox1* and the *35S::SoGA2ox2* cDNA fusion constructs were transformed into *Arabidopsis* and tobacco (*Nicotiana sylvestris*), respectively. These overexpression lines have typical GA-deficient phenotypes, such as low rate of seed germination, short hypocotyl, small rosette size, small and dark-green leaves, short stem, small root system, and late flowering. Leaves are short and curled. Internode length is also decreased. Transgenic lines are defective in floral development. Flowers of transgenic *GA2ox2* lines are smaller (60%) than those of wild type plants and their stamens and ovaries are not well developed in tobacco. These phenotypes can be partially overcome by treatment with GA_3. The more severe dwarfism, the less effective the treatment with GA_3 is. Most seeds (90%) of WT and transgenic *GA2ox1* lines have germinated 3 days after sowing on agar medium. However, transgenic *GA2ox2* lines have a lower germination rate (50%) than those of WT plants and transgenic *GA2ox1* lines in *Arabidopsis*. These results indicate that the SoGA2ox1 and SoGA2ox2 proteins are functional in *Arabidopsis* and tobacco plants, and that some of their functions are different, even though they have the same enzymatic activity. (Supported by USDA grant no. 2002-35304-12693 and DOE grant no. DE-FG02-91ER20021).

350 The Annexin 1 mediates salt and ABA signal transduction in Arabidopsis

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Most components of abiotic stress signaling were identified by genomic approaches. Although it is powerful, it is hard to isolate protein of which function is regulated by post-translational modification. We screened Arabidopsis root microsomal proteins that increase in response to salt treatment with proteomic methods, MALDI-TOF mass spectrometry following 2D electrophoresis. We identified the p34 protein which increases in membrane fraction after salt treatment is Annexin 1. Increase of Annexin 1 in membrane fraction is not a result of increase of gene expression, rather a result of translocation from cytosol to the membrane fraction. Annexin 1 translocation in response to salt occurs in time- and concentration-dependent manner. Mannitol, PEG and ABA also increase the translocation of Annexin 1 to the membrane fraction, suggesting that Annexin 1 acts as in abiotic stress signaling. Supporting this is the observation that T-DNA inserted mutant of annexin 1 showed suppression of seed germination on salt and Mannitol-containing media. The annexin 1 mutant also showed ABA hypersensitivity on seed germination.

351 Characterization of AtRNR2 and its interaction with COP9 Signalosome (CSN) subunit

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CSN7 is a component of the COP9 signalosome (CSN). csn7(fus5) mutants show a constitutive photomorphogenic (cop) phenotype common to all known mutations in the Arabidopsis CSN. As with other CSN components, CSN7 has a PCI domain, is present both in CSN-dependent and CSN-independent forms, and is detected both in the nucleus and in the cytoplasm. To further understand the role of CSN7 in regulating plant development, a two-hybrid screen using CSN7 as a bait was carried out. Seven positive CSN7 interacting clones were isolated. Two of those clones encoded other CSN subunits, while five encoded other proteins. The interaction sites of these proteins were mapped to one of three sections CSN7. This analysis pointed out that CSN7 interacts with other PCI-containing CSN subunits through its PCI domain, while other regions interact with the other proteins. One of the interactors was identified as the small subunit of the ribonucleotide reductase, RNR2, a component of the RNR holoenzyme, which is a key enzyme in dNTPs biosynthesis. In order to understand the biological significance of the interaction, a csn7 mutant specifically deficient in its ability to interact with RNR2 was isolated from a library created in a random mutagenesis method. During the work it was found out that in Arabidopsis, like in Saccharomyces cerevisiae, there are two different genes encoding the RNR small subunit. The two RNR?s were named RNR2a and RNR2b. Although the two proteins are highly similar, they differ in their mRNA expression pattern, in their interaction with CSN7 and in their ability to rescue the yeast RNR mutants.

352 Auxin status in the polar transport impaired *atmdr1-1* mutant

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Polar auxin transport (PAT) refers to the basipetal movement of auxin from its site of synthesis in the apex. This biased transport of auxin is facilitated by the basal localization of an efflux carrier complex containing PIN1. The ATMDR1 ABC transporter is necessary for normal levels of PAT. The T-DNA insertional mutant *atmdr1-1* exhibits an 85% reduction in auxin transport capacity. This mutation abolishes the basal localization of the PIN1 auxin efflux carrier which could explain the PAT phenotype. In order to probe the effect of the *atmdr1-1* mutation on auxin distribution we performed visual measurements of the auxin responsive promoter DR5 fused to GUS. Light grown seedlings displayed similar staining in the root and hypocotyl, however *atmdr1-1* cotyledons appeared faintly stained compared to the wild type. Either, this result indicated disrupted polar auxin transport (PAT) from the apex, through the petiole, to the cotyledons or a decrease in auxin sensitivity. A dose-response analysis was performed to determine the sensitivity of the tissue to exogenous auxin. The fluorogenic substrate MUG, combined with a fluorescence plate reader enabled quantitative assessment of DR5::GUS activity. The reporter was induced by treating the mutant and wild type with different concentrations of auxin ranging from .05-50μM. The induction experiment yielded two similarly sloped dose response curves. As the applied auxin dose increased to 10μM, both genotypes responded with a 47% increase in total signal. We hypothesize that the lower DR5 activity in mdr cotyledons is due to reduced auxin transport through the petiole from the shoot apex. Based on these results it was concluded that DR5::GUS staining in the cotyledons is a valid PAT assay. This procedure provides a tool to examine the mechanism of PAT and its dependence on proper PIN1 localization by testing various deficiencies and control elements for their effect on the system. These include not only the atmdr1-1 mutation, but also a large class of proposed synthetic and endogenous regulators.

353 ACD6 integrates light and salicylic acid signaling in defense response

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Signal transduction events control plant responses to environmental and endogenous stimuli. Plants can integrate multiple signals to activate unique responses, which may facilitate their adaptation to different conditions. The plant phenolic compound salicylic acid (SA) mediates responses to stress and pathogen attack, often through interactions with other signals, such as light or ethylene. We report here the isolation and characterization of Arabidopsis Accelerated Cell Death 6 (ACD6), a novel protein with putative ankyrin and transmembrane regions. The expression of ACD6 and the phenotypes of a dominant gain-of-function mutant *acd6-1* (spontaneous cell death, increased disease resistance and constitutively activated defenses) are light and SA dependent. Plants harboring *acd6-1* or overexpressing ACD6 have high SA accumulation, enhanced resistance to *Pseudomonas syringae*, and/or cell death formation. We showed previously that *acd6-1* conferred enhanced responsiveness to SA. We now find that plants lacking ACD6 have reduced responsiveness to an SA agonist and elevated disease susceptibility to *P. syringae* infection. Therefore, ACD6 integrates light and SA signaling to amplify defense signal transduction. Our work establishes a role for ACD6 in regulating defense responses and cell death in Arabidopsis.

354 ACD6, a novel ankyrin protein, integrates light and salicylic acid signalin

Hua Lu, Debra N. Rate, Jong Tae Song and Jean T. Greenberg
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Signal transduction events control plant responses to environmental and endogenous stimuli. Plants can integrate multiple signals to activate unique responses, which may facilitate their adaptation to different conditions. The plant phenolic compound salicylic acid (SA) mediates responses to stress and pathogen attack, often through interactions with other signals, such as light or ethylene. We report here the isolation and characterization of Arabidopsis Accelerated Cell Death 6 (ACD6), a novel protein with putative ankyrin and transmembrane regions. The expression of ACD6 and the phenotypes of a dominant gain-of-function mutant *acd6-1* (spontaneous cell death, increased disease resistance and constitutively activated defenses) are light and SA dependent. Plants harboring *acd6-1* or overexpressing ACD6 have high SA accumulation, enhanced resistance to *Pseudomonas syringae*, and/or cell death formation. We showed previously that acd6-1 conferred enhanced responsiveness to SA. We now find that plants lacking ACD6 have reduced responsiveness to an SA agonist and elevated disease susceptibility to *P. syringae* infection. Therefore, ACD6 integrates light and SA signaling to amplify defense signal transduction. Our work establishes a role for ACD6 in regulating defense responses and cell death in Arabidopsis.

355 Differential Temperature Sensitivity Distinguishes Two Arabidopsis Clocks

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Circadian rhythms are widespread in nature and reflect the activity of an endogenous biological clock. In metazoans, the circadian system includes a central circadian clock in the brain as well as distinct clocks in peripheral tissues such as the retina or liver. Similarly, plants have distinct clocks in different cell layers and tissues. We show that two different circadian clocks, distinguishable by their sensitivity to environmental temperature signals, regulate the transcription of genes that are expressed in the *Arabidopsis thaliana* cotyledon. One oscillator, which regulates *CAB2* expression, responds preferentially to light-dark versus temperature cycles and fails to respond to the temperature step associated with release from stratification. The second oscillator, which regulates *CAT3* expression, responds preferentially to temperature versus light-dark cycles and entrains to the release from stratification. Finally, the phase response curves of these two oscillators to cold pulses are distinct. The phase response curve of the oscillator component *TOC1* to cold pulses is similar to that of *CAB2*, indicating that *CAB2* is regulated by a TOC1-containing clock. The existence of two clocks, distinguishable on the basis of their sensitivity to temperature, provides an additional means by which plants may integrate both photoperiodic and temperature signals in order to respond to the changing seasons. This work was supported by grants (MCB-9723482 and MCB-0091008) from the National Science Foundation.

356 Isolation of Indole-3-Butyric Acid Response Mutants

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The ubiquitous phytohormone auxin exists endogenously in two forms, indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA). Genetic and biochemical studies suggest that IBA is converted to IAA using a mechanism similar to peroxisomal fatty acid β-oxidation. Mutants faulty in this conversion display enhanced root elongation and suppressed lateral root formation on exogenous IBA, but remain sensitive to IAA. In particular, mutants with defects in certain peroxisomal matrix or biosynthetic enzymes cannot β-oxidize lipids, and display both fatty acid and IBA related defects. Because seedlings require energy from lipid oxidation following germination, general β-oxidation mutants grown without supplemental sucrose in the dark exhibit a short hypocotyl. Other IBA-response mutants may possess different lesions that influence IBA transport, perception, or regulation. Mutagenized *Arabidopsis* seeds were screened on inhibitory IBA concentrations to isolate 19 new IBA-response mutants. These mutants were examined under different light, sugar, and hormone conditions and classified according to their defects in fatty acid and/or IBA β-oxidation. Recombination mapping and genetic complementation revealed that five mutants are alleles of previously identified IBA-response mutants, while 14 mutants may represent new loci. Future efforts will focus on using positional cloning to identify the genes defective in novel IBA-response mutants.

357 A novel mutation in the Brassica homolog of the green revolution Gene

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Dwarf genes have been successfully used to reduce plant height and their use was a major factor in the green revolution of wheat and rice. These dwarfs have mutations in the gibberellin (GA) pathway. It has been proposed that GA regulates stem growth by converting repressors of plant growth to nonrepressing forms allowing growth to occur. The repressors of stem elongation, GAI and RGA in Arabidopsis and their orthologs in wheat (Rht), maize (d8), grapevine (VvGAI), barley (SLN), and rice (SLR) are DELLA proteins. Mutations of the DELLA domain or nearby sequences in the N-terminus of these repressors generated dominant GA insensitive dwarfs. Recent studies show that GA derepresses growth by degrading the repressors and the DELLA domain is critical for this process. Although many studies have shown that the C-terminus of the repressor proteins contain the functional domain, a gain of function mutation causing dwarf phenotype generated by mutation in this end has not been reported. Using comparative mapping with Arabidopsis, we clone *Brassica RGA1* (*BrRGA1*) from *Brassica rapa* and we identify a novel mutation in the C-terminus of this gene causing a dominant GA insensitive dwarf. Expression studies show the *BrRGA1* is ubiquitously expressed in different tissues. Importantly, we find that the mutant protein is resistance to degradation by GA and IAA. The canola hybrids and inbreds containing *Brrga1* mutant gene show significant height reductions in field experiments. This dwarf mutant could be useful to gain more information on the GA pathway and to solve the lodging problems in canola fields.

358 Functional Characterization Of The Arc1-Related (*At*PUB) Family In *Arabidopsis*

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Arabidopsis contains a large number of U-box proteins (*At*PUBs) with many of these showing sequence similarity to *Brassica* ARC1. This amino acid similarity is found in the U-box region following by several arm repeats. *Brassica* ARC1 is an E3 ubiquitin ligase which act downstream of the S Receptor Kinase (SRK) to promote ubiquitination and protein degradation which in turn results in the rejection of self-incompatible *Brassica* pollen. As part of the characterization of the ARC1 related *At*PUBs, we have developed better algorithms to detect plant arm repeats and used these to identify arm repeat proteins in *Arabidopsis*. Phylogenetic analysis showed that the 108 predicted *Arabidopsis* arm repeat proteins can be divided into multiple clusters with wide differences in their domain organizations. Interestingly, 41 of the 108 *Arabidopsis* arm repeat proteins belong to the U-box *AtPUB* family and represent the largest class of *Arabidopsis* arm repeat proteins. We are interested in understanding the function of these ARC1 related *AtPUB* proteins in self-compatible *Arabidopsis*. Based on a phylogenic tree, representative members were selected from each subgroup for detailed analyses. Using RNA blot analysis, we have demonstrated that they are expressed in a variety of tissues in *Arabidopsis*. In addition, the selected *At*PUBs have been found to be functional E3 ubiquitin ligases. Finally, the yeast two-hybrid system has been used to analyze the interactions between the selected *At*PUBs and various *Arabidopsis* receptor kinases to determine if similar interactions to that of SRK and ARC1 exist in *Arabidopsis*. Our results indicate that the *At*PUBs interact specifically with S-domain receptor kinases. Thus, the ARC1 related *At*PUBs may be involved in a conserved receptor kinase signaling pathway regulating other aspects of plant growth and development.

359 Analysis of ETHYLENE INSENSITIVE6 and the ENHANCER OF ETHYLENE INSENSITIVITY

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The plant hormone ethylene regulates a variety of developmental and stress responses, including the triple response displayed by etiolated seedlings. We have previously identified *ETHYLENE INSENSITIVE6* (*EIN6*) in a genetic screen for mutants deficient in the triple response. Mutations in *EIN6* are epistatic to *CONSTITUTIVE TRIPLE RESPONSE* (*CTR1*) and therefore *EIN6* acts downstream of CTR1 in the ethylene signaling pathway. Further characterization of this mutant revealed that it contained a second recessive mutation, *ENHANCER OF ETHYLENE INSENSITIVITY* (*EEN*), which dramatically enhanced the *ein6* ethylene phenotype. In the absence of *een*, *ein6* plants display an ethylene insensitive root phenotype. Mutations in *EEN* show no ethylene phenotype on their own, but dramatically enhance the *ein6* ethylene insensitive root phenotype; the *ein6/een* double mutants show a near complete lack of the triple response. *EIN6* was mapped to the bottom of chromosome 3. Positional cloning of *EIN6* revealed that it encodes a DNA binding protein, consistent with its downstream position late in the ethylene signaling pathway. Previously it was noted that the *ein6/een* double mutant was hypersensitive to taxol (Philos Trans R Soc Lond B Biol Sci 350: 75-81), it has now been determined that the *een* mutation is the cause of drug hypersensitivity. This phenotype may also be associated with a recently published finding that *ein6/een* plants are affected in the expression of the mechanical stimulation response gene *TCH3* and act downstream of calcium (Plant Physiol 128: 1402-9). We are currently identifying new alleles of *EIN6*. We are also engaged in the positional cloning the *EEN* gene.

360 Characterization of a family of potential G-protein coupled receptors

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G- protein coupled receptors (GPCRs) compose a diverse family of membrane proteins responsible for the perception of a wide variety of signals in many eukaryotic organisms. They act in conjunction with heterotrimeric G-protein complexes to initiate cellular signaling cascades upon activation by extracellular ligands. While in plants the components of a heterotrimeric G-protein have been identified and G-protein mediated signaling pathways have been implicated in a number of physiological processes, only one strong candidate for a receptor has been identified thus far. As this dearth is in sharp contrast to the abundance of GPCRs observed in other eukaryotes, it seems likely that a number of receptors have been overlooked because of insufficient primary sequence similarity. To explore this possibility, we are pursuing the characterization of a family of membrane proteins in *Arabidopsis thaliana* through a reverse genetics approach, using putative roles as GPCRs as a paradigm for investigating their function. In addition, we are developing a yeast-based assay for plant GPCR function.

361 Mislocalization of the PIN1 auxin efflux protein and enhanced tropisms in MDR mutants

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Many aspects of plant growth and development are dependent on the basipetally-biased flow of the hormone auxin, as evidenced by the effects of mutations and pharmacological agents that impair it. Rectification of auxin transport in stems is believed to result from the basal localization within cells of the PIN1 membrane protein, which conducts efflux of the auxin anion. Recently, mutations in two multidrug resistance-like genes were shown to block polar auxin transport in the hypocotyls of Arabidopsis seedlings, indicating that MDR-type (p-glycoprotein) ABC transporters function in the PIN1-dependent polar auxin transport process. Here we show that the *mdr* mutants displayed enhanced gravitropism and phototropism instead of the impaired curvature development expected of mutants lacking polar auxin transport. Gravistimulated *mdr1* hypocotyls curved twice as much as wild-type seedlings. Kinetic studies showed that the greater curvature in *mdr1* seedlings was due to a doubling of the rate rather than a prolonging of the 2-h response time course. This implies that the auxin gradient across the hypocotyl induced by gravistimulation was twice as steep in the mutant, rather than twice as persistent. The impaired polar auxin transport and hypertropism phenotypes, which at first may seem contradictory, can be explained by the finding that the *mdr* mutations disrupted the special accumulation of PIN1 protein along the basal end of hypocotyl cells. In *mdr1* single mutants, PIN1 appeared generally delocalized and sometimes in punctate patterns. Plasma-membrane localization of the AHA2 proton pump was not affected by the mutation. In *mdr1 pgp1* double mutants, PIN1 was present along all sides of some hypocotyl cells without any apparent basal concentration. We conclude that as a consequence of disrupted PIN1 localization, lateral auxin conductance becomes a larger proportion of the whole in *mdr* mutants; loss of basipetal bias in auxin flow and greater growth differentials across the hypocotyl result. The mechanism by which MDR-like ABC transporters influence the subcellular localization of PIN1 is under investigation.

This work is in press in *Nature*.

362 Metabolism of the xanthophyll precursors of abscisic acid

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ABA is derived from xanthoxin by the cleavage of oxygenated carotenoids, also called xanthophylls. The isolation of mutants impaired in ABA biosynthesis, has proved to be particularly effective for cloning genes involved in most steps of the pathway. Zeaxanthin epoxidation leads to the formation of all-*trans*-violaxanthin. Yet evidence suggests that only *cis*-isomers of violaxanthin and neoxanthin are cleaved into xanthoxin by a family of 9-*cis*-epoxycarotenoid dioxygenases (NCED). Due to the absence of mutants impaired in these catalytic steps, the genes involved in the conversion of all-*trans*-violaxanthin to *cis*-xanthophylls remain to be characterized. We recently isolated a new ABA-deficient mutant of Arabidopsis, *Ataba4*, which is unable to synthesize *cis* and *trans* isomers of neoxanthin. Isolation of the *AtABA4* gene by map-based cloning is underway. In addition, we are characterizing a mutant affected in the *AtNCED6* gene which exhibits normal stature and tolerance to hydric stress, but presents seed specific phenotypes. The results of transcript and reporter gene expression analyses of this gene will be presented.

363 Gibberellin Biosynthesis and Response during Arabidopsis Seed Germination

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Hormone-mediated control of plant growth and development involves both synthesis and response. Previous studies have shown that gibberellin (GA) plays an essential role in Arabidopsis seed germination. In order to learn how GA stimulates seed germination, we carried out comprehensive analyses of GA biosynthesis and response using gas chromatography-mass spectrometry and oligo nucleotide-based DNA microarray analysis. In addition, spatial correlations between GA biosynthesis and response were assessed by in situ hybridization. We identified a number of transcripts, the abundance of which are modulated upon exposure to exogenous GA. A subset of these GA-regulated genes was expressed in accordance with an increase in endogenous active GA levels, which occurs just prior to radicle emergence. The GA-responsive genes identified include those responsible for synthesis, transport and signaling of other hormones, suggesting the presence of uncharacterised cross-talk between GA and other hormones. In situ hybridization analysis illustrated that expression of GA-responsive genes is not restricted to the predicted site of GA biosynthesis, suggesting that GA itself or GA signals are transmitted across different cell types during Arabidopsis seed germination.

364 Unique and overlapping functions of ARF and Aux/IAA gene families

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The plant hormone auxin regulates various aspects of plant growth and development. In order to understand the molecular mechanism of auxin-regulated gene expression, we are studying two related *Arabidopsis* gene families, the Aux/IAA and Auxin Response Factors (ARFs) whose members are involved in auxin signaling. The Aux/IAAs and ARFs proteins are transcriptional regulators. The Aux/IAAs appear to modulate auxin- mediated gene expression by interacting with the ARFs. The biological function for most of the Aux/IAA and ARF proteins is poorly understood. We have employed a reverse genetic strategy to deconvolute the role of the Aux/IAA and ARF transcription factors in auxin regulation. We have identified T-DNA insertion lines for almost 50 % of the Aux/IAAs and ARFs. However, most of these mutants failed to show obvious phenotypes, suggesting that functional redundancy may exist among the Aux/IAA and ARF gene family members. Therefore, we have generated double and triple mutants among various members of the gene families. A particular ARF double mutant shows a strong phenotype, including severely impaired lateral root formation and abnormal gravitropism in both hypocotyl and root. Recently, we are able to identify an ARF single mutant displaying a unique light related phenotype. Microarray analysis has provided unique insights into the molecular phenotype of these mutants.

365 The homeobox genes *ATHB7* and *ATHB12* are potential growth regulators of growth in response to drought in Arabidopsis

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In Arabidopsis HDZip proteins have been shown to act as mediators in reprogramming development in response to environmental conditions. Two of the proteins, ATHB7 and ATHB12, share more than 80% sequence identity in the deduced amino acid sequence of their HDZip motif. The transcript levels of both genes are induced several-fold by water deficit and by treatment with abscisic acid, consistent with a function in the plant drought stress response. The ABA induction of both genes is impaired in *abi1-1* and *abi2-1* mutant, background, suggesting both genes to be regulated by drought by a common mechanism. Translational fusions of the *ATHB7* and *ATHB12* promoters with the reporter gene GUS in transgenic Arabidopsis plants show activities of the two genes to be partly overlapping in elongating parts of the stems, rosette leaves and root tips. Based on the expression patterns and the phenotypes of transgenic plants that express *ATHB7* or *ATHB12* constitutively, we propose that the two genes may act as negative regulators of cell elongation in the shoot, rosette leaf and root in relation to water supply.

366 Localization of PIN proteins in flavonoid-deficient mutants

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Flavonoids comprise a diverse group of phenolic compounds many of which are biologically active and influence enzyme activity. Consistent with this, flavonoids localize in regions of state transition: the cotyledonary node, root/shoot transition zone, and distal elongation zone (DEZ)/root tip. In Arabidopsis, a number of flavonoid biosynthetic mutants, the *t*ransparent *t*esta (*tt*) mutants, are available for investigating the roles of different flavonoid species. *tt4* does not accumulate flavonoids; *tt7* accumulates kaempferol; and *tt3* accumulates excess kaempferol and quercetin. The *tt* mutants have a number of physiological phenotypes including longer primary roots and more secondary roots in seedlings grown without sucrose. In mature plants, differences in inflorescence length and time to flowering were found to be light reversible in all of the *tt* mutants examined except *tt4*. An apparent loss of apical dominance in inflorescences also occurs in *tt* mutants. Auxin transport profiles are altered in *tt4*, *tt7*, and *tt3*. As NPA binding sites in *tt4* mutants were not different from WT, we reasoned that the observed differences in auxin transport were due to altered localization of auxin efflux carriers, flavonoid interactions with a regulatory site, or some combination of these phenomena. Consistent with observed altered auxin transport profiles, the localization of the PIN proteins was altered in *tt4*, *tt7*, and *tt3* when compared to WT. PIN1 is mislocalized in *tt4* and altered in *tt7* and *tt3* root tips and DEZ. PIN2 localization in the root tip and DEZ in the mutants is similar to WT with subtle changes in *tt7*. PIN4 is mislocalized in the root tips and DEZ of *tt4* and *tt7*, and altered in *tt3*. Treatment with the auxin transport inhibitors NPA and TIBA also alter PIN1 and PIN4 localization in the root tips and DEZ of the mutants. Flavonoid precursor feeding experiments restore WT patterns of PIN1 and PIN4 localization in *tt4*; however, aglycone flavonol feeding resulted in altered localizations. Although some of the effects noted can be attributed to alterations in auxin bias, the majority cannot. These data suggest that certain flavonol species are required for correct polar targeting of PINs to cell membranes and support our hypothesis that aglycone flavonols act as autocrine effectors.

367 *SEUSS* is involved in auxin response

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The *seuss* mutant of *Arabidopsis* has been characterized as a floral mutant, and the novel *SEUSS* protein has been proposed to act as a transcriptional co-repressor (Bob Franks et al., Development, 2002). Most apparent in *seuss* mutant flowers are the apical split between carpels and the overall reduction in size. Here we report two new strong alleles of *seuss, seu-3* and *seu-4,* that have several hallmark phenes of auxin response mutants (*seu-3* has a nonsense mutation in the first exon, and is used as the reference allele for phenotypic characterization). In addition to disruption of gynoecium morphology and reduced seed set, *seu* has a subtle seedling phenotype. A small percentage of cotyledons are variably fused and have reduced vasculature. *seu* is also shorter than wild type with increased lateral branching. To determine whether *SEUSS* is involved auxin response, we examined *seu* roots. *seu* has fewer lateral roots, as well as longer primary roots and reduced gravitropism. When grown in culture with the exogenous auxin NAA, *seu* root growth is inhibited less than wild type, suggesting *seuss* is less sensitive to auxin. To determine specificity of *SEUSS*, crosses were made to various auxin and floral mutants. *seu* interacts synergistically with *ettin*, a gynoecium auxin response mutant, in both the flower and the root. Based on the above studies as well as other double mutant analyses, we propose that *SEUSS* may play a role in auxin reponse. Models for *SEUSS* action will be discussed.

368 Exploring the Importance of Histidine Kinase Activity to CKI1 Function

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Forty years after the discovery of the phytohormone cytokinin, the first putative cytokinin receptor, CKI1, was identified (1). The discovery of this histidine kinase-encoding gene, whose overexpression conferred cytokinin-independent responses on *Arabidopsis* callus tissue, paved the way for the ongoing dissection of the cytokinin signaling pathway in plants. Although CKI1 was originally implicated in cytokinin signaling, subsequent studies have shown neither cytokinin binding activity nor cytokinin dependent histidine kinase activity for CKI1. Recent work with null alleles of *CKI1* has shown that the *CKI1* gene product is essential for female gametophyte development in *Arabidopsis* (2). Specifically, cki1 female gametophytes do not progress beyond stage FG5 of female gametophyte development. This finding leaves open the possibility of a role for CKI1 in cytokinin signaling, as neither cytokinin levels within developing ovules nor the role cytokinins may play in female gametophyte development are currently known. Overexpression of *CKI1* in heterologous systems has been shown to activate cytokinin-inducible histidine kinase signaling pathways in a constitutive, rather than cytokinin-dependent, manner. To test the hypothesis that CKI1 is a constitutively active histidine kinase, we are using PCR-mediated targeted gene replacement to rescue sln1 lethality in yeast. This experiment further lends itself to the search for a ligand that will turn CKI1 activity on or off, as the case may be. While CKI1 may be able to function as a histidine kinase, histidine kinase activity may not be important for CKI1 function. In fact, recent work with ethylene receptors in *Arabidopsis* has shown that histidine kinase activity is not required for ethylene receptor signaling (3). To explore the link between histidine kinase activity and CKI1 function, we are investigating rescue of cki1 female gametophyte lethality with constructs likely to be deficient in histidine to aspartate signaling.

1) Kakimoto, T. (1996) Science, 274: 982-985.
2) Pischke *et al.* (2002) PNAS, 99: 15800-15805
3) Wang *et al.* (2003) PNAS, 100: 352-357

369 Regulation of IAA18 Gene Function

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Members of the AUX/IAA gene family modulate auxin signal response pathways in part through repression of auxin response factor (ARF) transcriptional activators. Gain-of-function mutations within domain II of several AUX/IAA genes disrupt auxin dependent, ubiquitin mediated proteolysis of these repressors, attenuating ARF dependent transcription from auxin inducible promoters. A Gain-of-function mutation within domain II of the IAA18 gene results in developmental phenotypes consistent with this regulatory model; mutant seedlings have altered cotyledon phyllotaxy and reduced vascular development, defects similar to seedlings homozygous for loss-of-function mutations in the ARF activator ARF5/MP. Thus, IAA18 protein may repress ARF5 dependent developmental programs. To gain insight into the regulation of the IAA18 gene product we are analyzing various reporters of IAA18 gene transcription and protein stability both in a developmental context and in response to induction by phytohormones and nutrients. In this way we hope to identify inputs which modulate ARF activator function.

370 Role of ethylene biosynthesis in hypocotyl gravitropism.

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Gravitropism is a plant's directional growth in response to gravity, exhibited as growth against gravity in stems and toward gravity in roots. During stem gravitropism, the asymmetrical distribution of the auxin causes differential growth and upward curvature while the gaseous hormone ethylene plays a modulating role in regulating the kinetics of growth asymmetries. Light also controls gravitropic curvature, potentially through its interaction with ethylene biosynthesis. The major goal of this project was to evaluate gene expression changes for members of the ethylene biosynthetic gene families *ACO* (1-aminocyclopropane-1-carboxylic acid [ACC] oxidase) and *ACS* (ACC synthase) after red-light treatment of dark-grown *Arabidopsis* seedlings. Seedlings were handled under a dim green light and red-treated seedlings were given a 6 min pulse with 11 μmol m^{-2} s^{-1} red light 18 hours prior to experimentation to be consistent with previous studies on the timeline of red-light inhibition of ethylene biosynthesis. T-DNA mutants for *ACS6* and *ACS10* exhibited statistically significant increases in mean gravitropic curvature compared to wild type seedlings by 3 hours after horizontal reorientation. This suggests that lowered ethylene level increases gravitropic curvature. Seedlings pulsed with red-light also exhibited enhanced gravitropic curvature. Relative RT-PCR was used to confirm a role for the red-light regulation of the different *ASC* genes. Preliminary evidence evaluated the expression for *ACS2, 4, 5, 6* and *EAT1* (which encodes an ACO), in total-RNA extracts of etiolated *Arabidopsis* seedlings, and suggests inhibition of *ACS4* after red-light treatment. Continued studies will evaluate the changes in key ethylene biosynthetic enzymes association with gravitropism and after light-pulse treatment.

371 Determination of Transcriptional Patterns Upon Glucose, Nitrogen Treatment in *Arabidopsis* Using the Full-Genome Affymetrix GeneChip

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Precise measurement of nutrients and adjustment of cellular activities accordingly are essential for plants and animals to survive. In response to continuous environmental and physiological changes, plants may use complex regulatory circuits to transmit sugar and nitrogen signals that ultimately affect gene expression. Understanding the molecular basis of nutrient signaling is critical for the improvement of crop vigor and productivity. Although it has been shown that both sugars and inorganic nitrogen are powerful regulators of gene expression, it is not known how widespread this phenomenon is on a global scale. Moreover, even though there has been a report on the effect of nitrate on gene expression profiling, only 5,500 genes were examined and the experiment was performed in the presence of sugar (Wang et al., 2000). To augment this study, we've conducted DNA microarray analysis using full genome Affymetrix GeneChips to measure transcriptional patterns of *Arabidopsis* in the presence of glucose, nitrate, or glucose and nitrate together. We have found that glucose is a much more potent regulator of transcription than nitrate. Glucose affected a broad array of genes associated cell growth and development, ranging from cyclins and transcription factors to transporters. Surprisingly, glucose was the primary signal in altering expression of genes associated with nitrogen metabolism other than genes associated with nitrate assimilation (which nitrate regulated). Moreover, there is a significant interaction between glucose and nitrogen signaling pathways, and sugar effects are often epistatic of nitrogen effects. This leads to a hypothesis that sugar has a dominant role in controlling carbon/nitrogen balance, a long known crucial factor for plant growth and development. We propose that nutrients such as glucose and nitrate have dual roles, both as metabolic components and as signals of metabolic status.

(Wang R, Guegler K, LaBrie ST, Crawford, NM (2000) Genomic analysis of a nutrient response in Arabidopsis reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. Plant Cell 12: 1491-1509)

372 Mechanisms of Glucose Signaling During Germination of *Arabidopsis thaliana*

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Glucose signaling, along with ABA signaling, has been implicated in regulating early plant development in *Arabidopsis*. High levels of exogenous glucose are thought to cause ABA accumulation, resulting in a delay of germination and an inhibition of seedling development. To test this hypothesis and decipher the complex interactions that occur during signaling, we determine the effects of sugar and ABA on one developmental event: germination. We show that levels of exogenous glucose lower than previously cited could delay the rate of seed germination in wild ecotype (WT) seeds. Remarkably, this effect could not be mimicked by an osmotic effect, and ABA was still involved. With higher concentrations of glucose, mutants *gin2* and *abi4*, previously identified as being glucose insensitive, exhibited germination kinetics similar to WT, indicating that glucose-insensitive phenotypes are not the same for all stages of growth and that signaling properties vary with glucose concentration. Higher concentrations of glucose were more potent in delaying seed germination. However, glucose-delayed seed germination was not caused by increased cellular ABA concentration, rather glucose appeared to slow down the decline of endogenous ABA. Except for the ABA insensitive mutants, all tested genotypes appeared to have similar ABA perception during germination, where germination was correlated with ABA dropping to a threshold level. In addition, glucose was found to modulate the transcription of genes involved in ABA biosynthesis and perception only after germination, suggesting a critical role of the developmental program. From our extensive analyses, we suggest that signals generated by osmotic stress, low concentration of glucose, high concentration of glucose, and high concentration of ABA are transmitted individually through a distinct pathway to control *Arabidopsis* seed germination.

373 Determination of biological functions of NPH3/RPT2: a plant-specific superfamily encoding putative scaffold proteins

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Organisms have co-evolved mechanisms of environment sensing and responsiveness. An example of such a mechanism is the formation of multimolecular complexes on scaffold proteins, increasing the speed and selectivity for specific signaling pathways that utilize molecules otherwise common to multiple pathways. Due to their sessile nature, plants have evolved specific signaling components to maximize developmental plasticity. In *Arabidopsis*, NPH3 and RPT2 are proteins involved in early phototropic responses and are members of a novel plant-specific superfamily composed of thirty-two members. These proteins share common features with mammalian and fungal scaffold proteins, such as the presence of multiple protein-protein interaction domains and association with sensor kinases, the phototropism photoreceptors. As part of NSF's *Arabidopsis* 2010, a multinational project to understand the function of all 25,000 genes in *Arabidopsis* by the year 2010, we are determining the biological functions of all members of the *NPH3/RPT2* gene family. In order to achieve our goals, we are determining protein expression profiles and generating loss- and gain-of-function mutants for each member. Protein expression analysis is being assessed in transgenic plants carrying translational fusions of NPH3/RPT2 proteins to GFP, driven by their endogenous promoter. By reverse genetics, we have identified potential loss-of-function mutant lines for 29 members of the family. Gain-of-function alleles have been transgenically generated by driving expression of family members from their native promoters fused to 4 transcriptional enhancer elements from the CaMV 35S promoter to maintain their spatial and temporal regulation. Putative overexpressor lines have been obtained for 28 of the 32.

374 The roles of Arabidopsis IAA-amino acid conjugate hydrolases in auxin homeostasis.

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Plants regulate auxin levels through complex interactions among *de novo* synthesis, degradation, influx, efflux, and conjugate synthesis and hydrolysis. A thorough knowledge of identity, regulation, and interaction of these pathways is key to understanding auxin influences on plant growth and development. We are using genetic approaches to understand the function and metabolism of auxin conjugates, particularly amide-linked conjugates of indole-3-acetic acid (IAA). Some IAA conjugates have auxin-like activity in bioassays. We have isolated mutants with altered responses to IAA-conjugates. *ilr1* (IAA-Leu Resistant) and *iar3* (IAA-Ala Resistant) are defective in amidohydrolases that cleave specific IAA conjugates to release free, active IAA. Based on similarity to ILR1 and IAR3, five more members of the Arabidopsis amidohydrolase gene family have been identified (*ILL1, ILL2, ILL3, ILL5 and ILL6*). We have compared the *in vitro* enzymatic activity of GST fusions of these hydrolases and found that four can hydrolyze IAA-amino acids. The expression patterns of promoter-GUS fusions suggest that the hydrolases have overlapping, yet distinct roles in plant development. We found that the triple hydrolase mutant, *ilr1 iar3 ill2*, displays several phenotypes suggestive of low endogenous auxin levels. Comparisons of the triple, double, and single hydrolase mutants have revealed the relative importance of each hydrolase in IAA-conjugate hydrolysis in seedlings. Interestingly, IAA-Ala may have auxin activity independent of hydrolysis because the triple hydrolase mutant remains sensitive to high concentrations of IAA-Ala, but is resistant to other conjugates. This work will aid in elucidating the roles of the amidohydrolase gene family and the IAA conjugates in auxin homeostasis.

375 *HIGHER RESPONSE TO ABA AT GERMINATION 1*, a novel gene involved in plant responses to water deficit and ABA

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Abscisic acid (ABA) is a major plant hormone that has been implicated in multiple developmental and physiological processes including seed development and germination, seedling growth, and plant responses to environmental stress (Bray, 2002. Plant, Cell and Environment 25: 153-161). Although as many as 38 different ABA response mutants have been identified in Arabidopsis, there is still need to identify additional components of ABA signaling in order to link the roles of the existing elements into cellular signaling pathways (Finkelstein *et al.*, 2002. Plant Cell Supplement: S15-S45). We have identified a new mutant, *higher response to ABA at germination 1* (*hrg1*), which exhibits hypersensitivity to ABA during germination and at the seedling stage. *hrg1* was identified in a gene-trap population during a screen to identify lines showing altered reporter-gene expression during water deficit. In the gene-trap line, β-glucuronidase (GUS) activity is down-regulated after water-deficit treatment. Down-regulation of GUS activity is also observed when seedlings are exposed to ABA or NaCl treatments. The gene trap *Ds* element (*DsG*) disrupts a gene that is predicted to encode a novel protein of 844 amino acids, and for which no functional domain can be identified. The *DsG* insertion is in the last of three exons of the gene, resulting in the creation of a transcriptional fusion between *HRG1* and *GUS*. Steady-state transcript levels of *HRG1* in wild type seedlings and the *HRG1-GUS* fusion in *hrg1* mutants are not altered by water deficit or ABA, suggesting the down-regulation of GUS activity in *hrg1* plants reflects translational or post-translational regulation. Mutant phenotypes have been detected in homozygous *hrg1* plants. *hrg1* seeds have increased dormancy and are hypersensitive to ABA application during germination. 8-day old seedlings are also more sensitive to ABA application since they accumulate higher levels of *RD19* transcripts than wild type when exposed to ABA. Characterization of *hrg1* and two additional allelic mutants will be presented. *h*

376 The SERK family of receptor-like kinases can homo- and heterodimerize at the plasma membrane

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The *Arabidopsis* Somatic Embryogenesis Receptor-like Kinase (SERK) family consists of five members: SERK1-5. All proteins display a similar structural organization with an extracellular domain connected by a single transmembrane domain to an intracellular serine/threonine kinase. The extracellular domains of the SERK family contain an N-terminal leucine zipper (LZ) followed by five leucine-rich repeats (LRRs) and a proline-rich sequence motif found in extensins and used as a hallmark to identify the SERK family in the *Arabidopsis* genome. The amino acid identity between the family members ranges from 89 to 77% in the extracellular domains and from 95 to 88% for the kinase domains. In order to understand whether the SERK proteins are functionally related we have tested if those proteins can interact with each other by expressing them as different green fluorescent protein (GFP) tagged protein variants in cowpea protoplasts. Using fluorescence energy transfer (FRET) measured by Fluorescence Lifetime Imaging Microscopy (FLIM) with the Time Correlated Single Photon Counting (TCSPC) technique we have demonstrated that at least three members of the SERK family are able to oligomerize in every possible dimeric combination at the plasma membrane. The oligomerization was always abolished when one of the receptor kinases was mutated. Our results could indicate that either those genes are functionally redundant or that in plants as in animals, heterodimerization among structurally related receptors is an important mechanism of diversifying downstream signaling pathways.

377 The role of the 5' untranslated region and polysome association in Blue High Fluence System mediated RNA destabilization

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Gene regulation at the post transcriptional level is largely a function of mRNA stability. The stability elements are known to be present on the 5' end or the 3' termini of the mRNA. Irradiation of etiolated *Arabidopsis* seedlings with a single pulse of blue light equivalent to 1 min of sunlight activates the Blue High Fluence (BHF) System and results in the destabilization of specific nuclear and plastid coded transcripts. These include specific members of the *Atlhcb1* gene family and *rbcL*. It is known that all of the sequences necessary for BHF-system mediated destabilization of the *AtLhcb1*3* RNA are located in the 64 base 5'UTR. Phototropin 1 is known to be the photoreceptor responsible for the BHF system; *Lhcb* transcript destabilization is absent in *phot 1* mutant seedlings. The 5'UTR is thought to direct the rapid and efficient formation of polysomes, thus preventing the degradation of the transcript by a cytoplasmically located RNase. To understand the role of phototropin and 5'UTR in the BHF system, polysomal association of the *Lhcb* transcript was checked in different transgenic lines, with or without the 5'UTR in WT or mutant *phot* backgrounds. Mono/polysomes were isolated by standard sucrose density gradient centrifugation from dark grown *Arabidopsis* seedlings, two hours after irradiation and RNA isolated. The seedlings were irradiated with a single pulse of blue light (10^4micromolm^{-2}) which activates the Blue Low Fluence (BLF) System or 10^5micromolm^{-2} which activates the BHF system. Northern analyses showed that seedlings receiving a pulse of blue light insufficient to activate the BHF-system show high levels of *Lhcb* transcript in the polysomal pool. Seedlings receiving a pulse of blue light to activate the BHF-system have background levels of *Lhcb* transcript in the polysomal and monosomal pools. The presence of the 5'UTR causes efficient formation of polysomes and the lack of it causes the transcript to be associated with the monosomes. The destabilization event appears to be localized in the cytoplasm.

378 Genetic Analysis of Circadian Period and Phase Determinants

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Rhythms with periods of approximately 24 hr are widespread in nature. Those that persist in constant conditions are termed circadian rhythms and reflect the activity of an endogenous biological clock. Although the clock is known to consist of a negative feedback loop, the components of the *Arabidopsis thaliana* clock have not all been identified, nor have the interactions among them been completely specified. Analysis of recombinant inbred lines between accessions Columbia and Landsberg *erecta* reveals much more variation in circadian parameters (period, phase, and amplitude) than is found in either parent. This is termed transgressive segregation and suggests that positive and negative alleles of multiple clock loci interact to determine clock characteristics. We have mapped Quantitative Trait Loci (QTL) for each clock parameter. Through loss of function and overexpression analysis, we have identified a number of loci that contribute to the establishment of period and phase and are candidate QTLs. Members of the *Arabidopsis Pseudo-Response Regulator* (*APRR*) family (Matsushika et al., 2000. Plant Cell Physiol. 41: 1002) appear to be potential and attractive candidates for each QTL identified. Specifically, T-DNA insertion alleles in *APRR3* and *APRR5* display short periods by leaf movement, while *APRR7* shows a long period phenotype. Interestingly, *APRR9* disruptants exhibit a normal period but a lagging circadian phase. *APRR9* is therefore required for proper circadian phase detemination, as we have shown previously for *phyB/oop1* and *cry1/oop2* mutants. This brings the number of loci affecting circadian phase to four: *PHYB* (Salomé et al., 2002. Plant Physiol. 129: 1674), *CRY1*, *SRR1* (Staiger et al., 2003. Genes Dev. 17: 256) and now *APRR9*. With a combination of loss and gain of function plants grown in various entraining regimes, we are now trying to determine the respective contribution of the APRRs, PHYB and CRY1 photoreceptors in the establishment of proper period and phase. This work was supported by grants from the National Science Foundation MCB-9723482 and MCB-0091008.

379 Cell Autonomy of the WD40 Protein TTG1

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The *Arabidopsis TRANSPARENT TESTA GLABRA1* gene encodes for a WD40 protein that is involved in the regulation of a variety of different processes including epidermal patterning in root, hypocotyl and leaf as well as seed coat development and synthesis of anthocyanins and condensed tannins. In contrast to its interacting partner *GL3*, a bHLH gene, recent studies indicate that *TTG1* functions in a non-cell-autonomous manner in trichome development and anthocyanin biosynthesis. We have constructed two different MYC-tagged *TTG1* versions that can rescue the anthocyanin deficient phenotype of a *Matthiola incana ttg1* mutant, when they are expressed under 35S promoter. These two versions and a combination of both will be expressed in *Arabidopsis* roots under the non-hair-cell specific *GL2* promoter to determine cell-autonomy of the TTG1 protein by examining its presence in the neighbouring root hair bearing cell-files. Control experiments will be performed with a cell-autonomous *MYC::GL3* construct.

380 Attachment of a Terminal GlcNAc Modification by *SPY* and *SEC*

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The goal of our work is to understand the function of *SPINDLY* (*SPY*) and *SECRET AGENT* (*SEC*) in Arabidopsis. *SPY* and *SEC* have extensive amino acid sequence similarity to mammalian O-linked N-acetyl glucosamine (GlcNAc) transferases (OGTs). Genetic evidence has shown that *SPY* is a negative regulator of gibberellin signal transduction. In addition, studies have shown *SPY* also participates in pathways controlling flowering and hypocotyl length. While *sec* plants do not exhibit pronounced phenotypes, loss of both *spy* and *sec* function causes lethality. These results indicate that *SPY* and *SEC* have overlapping functions and that OGT activity is essential in plants. Like animal OGTs, *SPY* and *SEC* are nucleocytoplasmic glycosyltransferases and catalyze the addition of a single GlcNAc in an O-linkage to the hydroxyl groups of serine and threonine residues. In addition, plant O-linked GlcNAc transferases (OGTs) are also capable of auto-glycosylation. We have used a Gal B (1-4) galactosyl-transferase (GalT) assay, which attaches [^{3}H]-galactose to terminal N or O-linked GlcNAc modifications, to show that SEC attaches a terminal GlcNAc modification to itself. Further, it has been shown through analysis of enzymatic and chemical treatments that the GlcNAc is attached to SEC with an O-linkage. Previously, we have also shown through the use of a GalT assay that SPY is able to auto-glycosylate. This work shows that the SPY terminal GlcNAc modification, like SEC's, is O-linked. After labeling with GalT, SPY was treated with the enzyme PNGase F which removes sugars from a protein if they are attached with an N-linkage or alkaline hydrolysis which removes sugars from a protein if they are attached with an O-linkage. After enzyme and chemical treatments, column chromatography analysis showed that labeled sugar was not removed from the protein after PNGase F treatment, but labeled sugar was removed from the after alkaline hydrolysis. This indicates that the GlcNAc modification which is attached to SPY occurs via an O-linkage.

381 CTR1 protein interactions suggest a model for the regulation of CTR1

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In *Arabidopsis*, ethylene signaling involves five ethylene receptors that bear strong homology to prokaryotic two-component receptors. Acting downstream of the receptors is the CTR1 Raf-like protein kinase. The N-terminal domain regulatory domain of CTR1 has been shown to physically interact with two of the ethylene receptors, ETR1 and ERS1, in yeast and in vitro. We have carried out further characterization of the interaction between CTR1 and the receptors in order to further understand its regulation *in vivo*. We first localized a region in the CTR1 N-terminal domain that is required for the interaction with ETR1 and ERS1. This region contains the site of the missense mutation in *ctr1-8*, which confers constitutive ethylene responses in the plant. We showed that the *ctr1-8* mutation disrupts the association with ERS1 as previously shown for ETR1. We also found that the CTR1 N-terminal domain interacts with the CTR1 kinase domain *in vitro*, and we postulate that this interaction may be involved in repressing CTR1 kinase activity as shown for some Raf kinases. This interaction between the CTR1 N-terminal domain and the CTR1 kinase domain is unaffected by the *ctr1-8* mutation. We have also demonstrated a role for phosphorylation in the activation of CTR1. Taken together, this data suggests a model for the regulation of CTR1 involving proteininteractions.

382 Functional heterogeneity of the 26S proteasome RP subunits

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Development and survival of all organisms in an ever-changing environment depends on an intricate interplay of synthesis and breakdown of signaling proteins. The most elaborate proteolysis machinery in eukaryotes is the ubiquitin/26S proteasome pathway. Regulated proteolyses by this route starts with the covalent attachment of a polyubiquitin chain to a target, a reaction regulated by the concerted action of a series of enzymes including the ubiquitin ligases that control target recognition. Polyubiquitinated proteins are then degraded by the 26S proteasome, a multisubunit protease, composed of two subcomplexes: the proteolyticaly-active core particle and the regulatory particle.

The regulatory particle (RP) is essential for target recognition. Originally, it was thought that the RP simply acts as a receptor for the polyubiquitin chain attached to target proteins. Recent studies, however, have revealed a more complex picture of RP function, suggesting that different subunits might be specific docking sites for non-overlapping sub-sets of targets. Here, we outline three examples indicating that factors other than the polyubiquitin chain are mediating proteasome/target interactions. Firstly, loss of function of the RP subunit RPN10 resulted in the stabilization of the ABA signaling protein ABI5, but did not affect the degradation rates of other proteasome targets like HY5 and PHYA [1]. Secondly, a recessive mutation that affects the RPN12a subunit led to a different phenotype compared to *rpn10-1*[2]. A third example addresses the additional complexities derived from the existence of multiple subunit isoforms. Twelve out of seventeen RP subunits in Arabidopsis are encoded by gene pairs. Characterization of the isoform pair RPN5a and b showed that these 94% identical proteins are not functionally redundant. Notably, these isoforms seem to act as antagonists throughout plant development. We hypothesize that Arabidopsis employs a pool of functionally distinct proteasomes to control its life cycle and responses to the environment.

1. Smalle et al. (2003) The Plant Cell 15 (4), 965-980.
2. Smalle et al. (2002) The Plant Cell 14 (1), 17-32.

383 Auxin and *STY* genes in Arabidopsis gynoecium development

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Gynoecia of the Arabidopsis loss-of-function mutant *sty1-1* display an abnormal style morphology and altered vascular patterning. These phenotypes, which are enhanced in the *sty1-1 sty2-1* double mutant, suggest that polar auxin transport (PAT) or auxin signalling might be affected by mutations in the related genes *STY1* and *STY2*. Transient chemical inhibition of PAT severely affects the apical-basal patterning of the gynoecium as do mutations in the auxin transport/signalling genes *PID, PIN1* and *ETT*. By transient treatments with the PAT inhibitor 1-N-naphtylphtalamic acid (NPA), we show that the apical-basal patterning of *sty1-1* and *sty1-1 sty2-1* gynoecia is hypersensitive to reductions in PAT and that *sty1-1* enhances the PAT inhibition-like phenotypes of *ett-1* and *pid-8* gynoecia, and of *pid-8* flowers. Furthermore, the *sty1-1* style phenotype is enhanced in the *pid-8* mutant background and in plants mutant for the E3 ligase-complex modulator *AXR1*. *STY1* and *STY2* are active not only in gynoecia but also in root primordia and root tips. In *35S::STY1* seedlings the number of lateral roots were significantly reduced compared to wild-type. As exogenous auxin restored the lateral root production to that of wild-type, the level or distribution of auxin, and not the auxin sensitivity or perception, in *35S::STY1* roots appears suboptimal. Our results indicate that the *STY* genes might affect auxin transport or signalling in specific tissues of the wild-type.

384 Control of the DWARF4 gene expression in the BR biosynthetic pathways

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The plant steroid hormones Brassinosteroids (BR) control many different aspects of plant growth and development, such as cell elongation, cell division, senescence, stress tolerance, and vascular system differentiation. Mutants defective in BR biosynthesis or signaling display severely retarded growth patterns regardless of their growing in the light or dark, indicating that BRs are generalized growth promoters. Of the enzymes involved in BR biosynthesis, *DWF4* mediates a rate-limiting step which is the 22ˆ-hydroxylation in the biosynthetic pathways. Thus, it was hypothesized that DWF4 expression pattern could represent the tissues of BR biosynthesis in Arabidopsis. Different methods including an RNA gel blot analysis, RT-PCR, and *DWF4::GUS* histochemical analysis consistently revealed that the *DWF4* transcripts differentially accumulate in the actively growing tissues, such as root tips, bottom of floral organs, axilary buds, collets, and elongating zone of dark-grown seedlings. Consistently, examining the endogenous levels of BR biosynthetic intermediates showed that metabolic flux after DWF4-mediated step increased in these tissues. Histochemical analysis of *DWF4::GUS* transgenic plants displayed that exogenous application of BRs and 2,4-D antagonistically regulate the DWF4 expression: decrease by BRs but increase by 2,4-D. Similarly, *DWF4::GUS* expression was up-regulated in the BR-deficient dwarf mutant background, however, the change was not noticeable or even weaker in auxin resistant mutants. When the *DWF4::GUS* construct was introduced into two brassinazole-resistant mutants*bzr1* and *bes1*, the GUS expression was effectively eliminated, suggesting that the BZR proteins act as negative regulator of the *DWF4* gene expression. Taken together, it is likely that controlling the *DWF4* expression may serve as a focal point in a cross talk with auxin as well as feedback down-regulation of BR biosynthetic genes.

385 The role of TRIPTYCHON and CAPRICE in generating the Spacing Pattern of Trichomes on Arabidopsis leaves

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The distribution of leaf hairs (trichomes) on the leaves of Arabidopsis shows a non-random spacing pattern which serves as a good model system to study the mechanisms underlying such patterning processes. Several mutants involved in this process have been studied in the past and their corresponding genes were cloned. All of these known genes encode putative transcription factors of the MYB or MYC class with the exception of Transparent Testa Glabra1, which encodes a protein with homology to WD-40 repeat containing proteins. We have analysed the role of these genes in trichome patterning by an expression analysis in different mutant backgrounds and assessed their intracellular localization and mobility. Our results are consistent with a model in which trichomes are selected from initially equivalent cells by a mechanism involving lateral inhibition through TRIPTYCHON and CAPRICE by non-autonomous cell-cell signaling.

Key words: Trichomes, Arabidopsis, Spacing pattern, Lateral inhibition, non-cell autonomous cell-cell signalling

386 Arabidopsis DRGs, a small family of highly conserved G proteins

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All eukaryotes appear to contain at least one DRG1 ortholog and one DRG2 ortholog. Amino acid sequence identities between animal, yeast and plant DRG2 proteins is about 66%, whereas identity between DRG1 proteins is about 71%. All Archaea appear to have one DRG-like protein that shares about 45% identity with eukaryotic DRGs. This level of conservation suggests that DRGs perform some basic and essential cellular function. Other G proteins function as molecular switches and participate in, for example, signal transduction, protein synthesis, vesicle transport, organization of the cytoskeleton and transport through nuclear pores. Little is known about the cellular functions of DRGs. Arabidopsis contains 3 DRG genes, which we call AtDRG1 (At4g39520), AtDRG2 (At1g17470) and AtDRG3 (At1g72660; AtDRG2 and AtDRG3 proteins are 95% identical). We have studied the normal expression patterns of each gene by analyzing promoter-GUS fusions in transgenic plants. Patterns of AtDRG1 and AtDRG2 expression were similar but not identical. For example, AtDRG2 is strongly expressed throughout seedling roots whereas AtDrg1 is expressed near the root apex and at the sites of lateral root initiation. Both of these genes were expressed in leaves and in several floral organs (stigma, receptacle, anther wall, pollen, silique wall, funiculus), but the timing of appearance and level of expression was distinct for each gene. Expression was not seen in seeds or embryos. AtDRG3 expression was undetectable under most conditions. Recent experiments suggest that AtDRG3, but not AtDRG1 or AtDRG2, may be regulated by water stress. These expression patterns will help to identify the tissues and organs whose growth might be affected by loss-of-function mutations in each gene. LOF mutations in AtDRG1 and AtDRG2 have been generated by T-DNA insertions (AKF and Salk collections) and by RNA interference. Single mutants appear to have only small effects of plant morphology. Double mutants are being generated. Supported by NIH (GM54276) and the Plant Molecular Biology Center, NIU.

387 The GA-Response Gene *SLY1*, a putative F-box subunit of an SCF E3 ubiquitin ligase

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The *SLEEPY1* (*SLY1*) gene of Arabidopsis is a positive element of the GA signaling pathway. Recessive mutations in SLY1 result in all of the phenotypes expected of a GA-insensitive mutant including increased seed dormancy, reduced fertility, and growth as a dark green dwarf. We cloned *SLY1* by map based cloning to elucidate the mechanism by which *SLY1* controls the GA-response pathway. *SLY1* encodes a 151 amino acid protein with a consensus F-box domain. This result suggests that SLY1 is the F-box subunit of an SCF E3 ubiquitin ligase controlling GA response. Interestingly, mutations in *SLY1* lead to increased accumulation of the RGA (repressor of ga1-3) protein, a member of the DELLA family of putative transcription factors. RGA is a negative regulator of GA signaling that appears to be subject to GA-regulated protein degradation. This suggests that GA may target RGA for degradation via SCF^{SLY1} mediated ubiquitylation. This would make SLY1 a negative regulator of a negative regulator of GA signaling. Arabidopsis has a large number of F-box genes, but only one other is similar to SLY, hence we have named it SLY2. We are investigating if this is gene also involved in the GA response pathway.

388 New ethylene insensitive mutants affected in tryptophan biosynthesis

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Exposure of dark-grown Arabidopsis seedlings to ethylene leads to characteristic morphological changes collectively known as the triple response. The phenotypic effects of ethylene in seedlings roots (i.e. dramatic inhibition of root elongation and an increase in the number of root hairs) are known to require normal levels of auxin signaling and response. We are interested in understanding the mechanisms of the ethylene-mediated root shortening and, in particular, the role of ethylene-auxin crosstalk in this process. Towards this goal, we isolated several mutants that are specifically insensitive to ethylene in roots, yet display normal gravity and wild-type sensitivity to auxin.

Positional cloning of *wei2* revealed that this gene encodes ANTHRANILATE SYNTHASE ALPHA SUBUNIT1 (ASA1). ASA1 catalyzes the conversion of chorismate to anthranilate (i.e. the rate-limiting step in tryptophan (trp) biosynthesis). Trp and its biosynthetic intermediates, in turn, can serve as precursors of auxin. Consistent with this notion, the phenotypic defect of *wei2* in ethylene can be fully or partially complemented by the exogenous addition of trp or auxin, respectively.

Microarray experiments followed by the histochemical analysis of an *ASA1* promoter-*GUS* fusion revealed that *ASA1* is induced by ethylene in the root tip and its expression/induction largely coincides with that of the synthetic auxin reporter DR5. The analysis of DR5 in *wei2* and other ethylene-insensitive backgrounds is currently underway.

In addition to *wei2*, several "classical" trp-deficient mutants (*trp1*, *trp2* and *trp3*) also possess ethylene insensitivity in roots, yet are significantly more sensitive to ethylene than *wei2*. Conversely, *wei7*, another novel ethylene mutant that can be fully rescued by trp, is phenotypically indistinguishable from *wei2*. Preliminary data indicate that *wei7* is caused by a defect in the beta subunit of anthranilate synthase, ASB1, that along with ASA1 converts chorismate to anthranilate. Double mutant analysis between *wei2* and *wei7* is underway.

In summary, these results suggest the involvement of trp biosynthesis and, possibly, auxin in the ethylene response in seedlings roots. Our current model of the crosstalk between ethylene and auxin in root elongation will be discussed.

389 Systematic analysis of the ethylene response in segregating populations of *Arabidopsis thaliana* hybrid accessions

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Determining the functions of all 29,000+ Arabidopsis genes will require the use of a combination of different experimental approaches. Systematic identification of knockouts and classical forward genetic screens have proven to be extremely powerful for the analysis of gene function. However, not all gene functions can be easily uncovered using these techniques due to, for example, gene function redundancy, reduction or complete loss of function of the allele in the "wild type" accession, etc. The exploitation of the natural genetic variation promises to be a complementary tool in the hunt for gene function in Arabidopsis.

In the search for quantitative as well as qualitative phenotypic effects of natural allele combinations, we have obtained more than 200 F2 hybrid populations from diallel crosses of 21 *Arabidopsis thaliana* accessions. Preliminary analysis of the ethylene response of these hybrid populations indicates that both qualitative and quantitative transgressive segregation are common in these populations. Transgressive phenotypes restricted to a particular accession combination (i.e. ACCESSION 1 x ACCESSION 2) as well as those shared by all populations with a common parent (i.e. ACCESSION 1 x ANY OTHER ACCESSION) have been observed.

Segregation analysis of qualitative ethylene insensitive phenotypes as well as of albino seedlings suggest a relatively simple genetic architecture of these traits. Our findings open the possibility of identifying the genes responsible for the defects using classical recombination-based positional cloning approaches. Our progress in the genetic characterization of these traits will be presented.

390 RTE1 encodes a novel regulator of the ETR1 receptor

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Ethylene is a gaseous plant hormone that has profound effects on plant growth and development. Genetic analysis has been central in the elucidation of the ethylene signaling pathway, made possible through screens for both insensitive and constitutive response mutants. Ethylene-insensitive mutants fail to exhibit the classic triple response displayed by *Arabidopsis* seedlings grown in the dark in the presence of ethylene. The *etr1-2* mutant receptor confers weak ethylene insensitivity, and unlike other insensitive alleles can still bind ethylene. We utilized *etr1-2* in a screen for new components in the ethylene signaling pathway, and identified the *REVERSION-TO-ETHYLENE -SENSITIVITY-1 (RTE1)* locus. Loss of *RTE1* function results in suppression of the *etr1-2* insensitive phenotype, but is unable to suppress the insensitivity of stronger dominant insensitive mutants. Single mutant analysis revealed a phenotype reminiscent of *ETR1* loss of function mutants. Further analysis of double mutants suggests that loss of *RTE1* results in a non-functional *ETR1*. We cloned *RTE1* by positional cloning and found it to encode a novel integral membrane protein with homologues in other plants and also animals. RTE1 has a single homologue in *Arabidopsis* , which we have named *RTE2*, and which we speculate may have a redundant function. Sequence analysis reveals two regions of conserved cysteine and histidine residues which are common in metal binding proteins; one allele of *RTE1* has a point mutation at one of these cysteine residues. In addition, RTE1 shows weak homology to some metal binding enzymes. One hypothesis is that RTE1 may be important in binding metal ions, more specifically that it may be involved in the binding or association of the copper cofactor which is essential for ethylene binding.

391 Disruptions in AUX1-dependent auxin influx alter phytochrome A-dependent modulation of phototropism

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A key component of our current working model for phototropism is that the plant hormone auxin acts as the dominant regulator of the differential growth response leading to phototropic curvatures. Support for this contention comes from the finding that mutations in the *NPH4* locus, which encodes the auxin-responsive transcriptional activator ARF, result in seedlings whose hypocotyls fail to bend towards low-fluence rate blue light. *nph4/arf7*-null mutants do however exhibit a partial response to the same blue light stimulation if preirradiated with red light. This requires the red/far-red receptor phytochrome A and is proposed to activate a partially redundant ARF system. We have identified recessive second-site mutations in four loci that prevent this phyA-dependent recovery of phototropism. One of these loci, *MAP1* (*MODIFIER OF ARF7/NPH4 PHENOTYPES 1*), encodes AUX1, an apparent auxin influx carrier. On their own, *aux1* mutations, including *map1/aux1-201*, confer root-specific phenotypes, a finding previously attributed to the predominantly root-localized expression of *AUX1*. How therefore can we explain the influence of the *map1/aux1-201* mutation on the *nph4/arf7* hypocotyl phenotype? One clue comes from study of a transgenic line carrying an AUX1::GUS translational fusion, in which we found that AUX1 is expressed in both roots and hypocotyls of dark-grown seedlings. Thus at a minimum AUX1 and NPH4/ARF7 are co-expressed in the affected phototropic organ. Additional experiments are currently underway to analyze the affects of other *aux1* alleles on the *nph4/arf7* phototropic phenotype and to determine precisely how AUX1 influences phyA-dependent modulation of phototropism.

392 Multiple photoreceptor modulation of phototropin 1-induced phototropism and the role of nuclear events

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The complex wavelength dependence governing phototropism in young seedlings suggests that multiple signaling pathways are functioning to control this seemingly simple physiological response. It is now accepted that the phototropins (phot1 and phot2) function as partially redundant receptors of directional blue light (BL) cues necessary to induce phototropism. We have used Arabidopsis lines that are singly and multiply mutant for non-phototropin receptors to show that at least four photoreceptors - phytochromes A and B (phyA and phyB), and cryptochromes 1 and 2 (cry1 and cry2) - function as "secondary" BL receptors in the modulation of phot1-dependent phototropism. PhyA appears to function as a major positive modulator, while phyB plays only a minor role, although both agonist and antagonist functions were observed. Cry2 appears to function additively with phyA as a major positive regulator, while cry1 appears to act negatively. While little is know about how the cryptochromes signal, a number of phytochrome signaling intermediates have been discovered. We have examined the phototropic response in a number of phyA-signaling mutants and have found that mutations in the *EID1*, *FHY1/PAT3*, *FHY3*, and *SPA1* genes each alter phot1-dependent phototropism. Significantly, each of the proteins encoded by these genes is nuclear localized, implying that phyA is influencing phot1-dependent phototropism via nuclear events. This is an exciting possibility given that we have previously tied phototropic responsiveness to changes in gene expression occurring via activity of an auxin-responsive transcriptional activator, NPH4/ARF7, and further suggest a cooperative interaction between auxin- and phyA-modulated transcriptional activities.

393 MADS-box protein, AGL24 is specifically bound to and phosphorylated by Meristematic Receptor-Like Kinase (MRLK).

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Intercellular signaling mediated by receptor-like kinases (RLKs) is important for diverse processes in plant development, although downstream intracellular signaling pathways remain poorly understood. Proteins interacting directly with RLK were screened for by yeast two-hybrid assay with the kinase domain as bait. A MADS-box protein, AGL24 was identified as a candidate substrate of MRLK (Meristematic Receptor-Like Kinase), which was named for its spatial expression in shoot and root apical meristems in Arabidopsis. The AGL24 protein specifically interacted with and was phosphorylated by the MRLK kinase domain in in vitro assays. The simultaneous expression of *AGL24* and *MRLK* in shoot apices during floral transition suggested that the interaction occurs in plants. Using plants constitutively expressing a fusion protein of AGL24 and green fluorescent protein, the subcellular localization of AGL24 protein was observed exclusively in nucleus in apical tissues where *MRLK* was expressed, while AGL24 was localized in both cytoplasm and nucleus in tissues where no *MRLK* expression was detectable. These results suggest that *MRLK* signaling promotes translocation of AGL24 from the cytoplasm to the nucleus. We have also generated the transgenic plants which overexpress *AGL24* to investigate the function of *AGL24*. These plants showed early flowering phenotype as reported by Michaels *et al.* (Plant J. 2003), indicating that *AGL24* acts as a promoter of flowering. We propose that the RLK signaling pathway involves phosphorylation of a MADS-box transcription factor.

394 Type-A ARRs are redundant negative regulators of cytokinin signaling

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Arabidopsis Type-A Response Regulators (ARRs) are a family of ten genes that are rapidly induced by cytokinin and are highly similar to bacterial two component response regulators. These ten genes are highly conserved in the receiver domain and their sequences fall into five most similar pairs. We have isolated T-DNA insertions in all the type-A ARRs and constructed multiple insertional mutants up to the *arr 3,4,5,6,8,9* hextuple mutant which comprises three of the five most similar pairs. We performed various cytokinin response assays on the *arr* mutants, including primary root elongation, lateral root formation, shoot initiation and leaf senescence. Single *arr* mutants were indistinguishable from the wild type. Double and higher order *arr* mutants showed increasing sensitivity to cytokinin, indicating functional redundancy among type-A ARRs and that these genes act as negative regulators of cytokinin responses. Different *arr* mutant combinations contributed to altered cytokinin responsiveness in different assays with varying strengths, suggesting some specificity in this gene family. We examined the expression of cytokinin upregulated genes in *arr* mutants on exogenous cytokinin application. Cytokinin induction of these genes were amplified in *arr* mutants, indicating that the primary response to the cytokinin signal is affected. In addition, *arr* mutant seedlings showed compromised responses to redlight and adult plants developed elongated petioles under short day conditions. Further results from analyses of the *arr* mutants will also be presented. In sum, our data suggests that type-A ARRs are partially redundant and play a negative role in cytokinin signaling.

395 Homo- and Heterodimeric Interactions Among the Arabidopsis 1-Aminocyclopropane-1-Carboxylic Acid Synthase Gene Family Members

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1-Amino-cyclopropane-1-carboxylic acid (ACC) synthase (ACS) is one of the key enzymes in the ethylene biosynthetic pathway. The completion of the Arabidopsis genome sequence by the Arabidopsis Genome Initiative (AGI) revealed the presence of twelve putative *ACS* genes, *ACS1-12*, dispersed among the five chromosomes. *ACS1-5* have been previously structurally characterised, and two of them *ACS1* and *ACS3* are pseudogenes. Complementation analysis with the *E. coli* aminotransferase auxotroph DL39, reveals that *ACS10* and *12* are aminotransferases and the remaining eight genes encode authentic *ACS*es that represent the Arabidopsis ACSome. All genes ,except *ACS3*, are transcriptionally active and differentially expressed during Arabidopsis growth and development.

ACS is a homodimer with shared active sites and the question arises whether the subunits of the various isoenzymes have the capacity to heterodimerize. To address this question, we have carried out fuctional intermolecular complementation experiments in *E.coli* JAde6 by coexpressing the ACS K278A and ACS Y92A mutants of each isoenzyme (homo) or from different isoenzyme (hetero). The results show that all isoenzymes homo- and heterodimerize. However, functional heterodimers are observed only among members that belong to the same phylogenetic branch. The inactivity of certain heterodimers is not due to the absence of heterodimerization but rather to a structural restrain that prevents the shared active sites to be functional.

396 Screening of hormone response mutants from GFP enhancer trap lines

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Many hormone response genes have been defined by genetic screens. However, it is possible that there are genes that play an important role for a regulation of a hormone response that may not show the expected phenotypes in genetic screens such as an altered response to exogenously applied hormone. To identify new genes in hormonal response pathways in *Arabidopsis* we are using a GFP (green fluorescent protein) reporter gene enhancer trap system to speficially identify genes that are up or down regulated in response to hormone application. The screen involves moniting GFP expression changes from enhancer trap lines after the addition of ABA, GA, ethylene or auxin. In this poster we have focussed on the ABA reponsive class. To date, we have screened 434 enhancer trap lines and have identified 12 independent lines that show interesting patterns in response to ABA addition. One line exhibited an expression pattern that is suppressed within 24 hours of ABA addition. Furthermore, this pattern change is dependent on ABA concentration. Another line, which shows induction in vascular tissue by ABA, also showed an ABA response defect at the level of germination. Specifically, cotyledon expansion was inhibited by ABA or pacrobutrazol, the GA biosynthesis inhibitor. Together with the expression pattern data, it appears the target gene identified in the enhancer trap screen is required for ABA responsiveness in the seed. We will report the GFP expression patterns and identification of the targeted genes of these lines.

397 Functional Analysis of KAPP in Arabidopsis

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Kinase Associated Protein Phosphatase (KAPP) is the only phosphatase that has been shown to physically interact with multiple receptor like protein kinases (RLKs). KAPP is a unique multidomain protein, it contains a type I membrane anchor at its N-terminus, a forkhead-associated (FHA) domain in its central part, and a type 2C phosphatase domain at its C-terminus. To understand the role of Ser/Thr phosphatase-KAPP in coordinating RLK signaling, we carried out detailed functional analyses of KAPP in Arabidopsis. Our data showed that all three domains of KAPP are functional in vivo. Transgenic analysis of KAPP (D346G) overexpression lines suggests that KAPP (D346G) has created a dominant negative effect, confirming previous data that KAPP has physical interactions with multiple RLKs in vitro/in vivo. Further genetic studies indicate that FHA domain mediated protein-protein interaction is responsible for this dominant negative effect. Using TAP-tagged KAPP as bait, we are conducting proteomic analysis to dissect KAPP-involved protein complexes, which will yield further insights into the molecular mechanisms of KAPP-mediated RLK signaling pathways.

398 Structure-function analysis of BRI1 reveals its activation mechanism

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Brassinolide Insensitive 1 (*BRI1*) plays an essential role in plant growth and development as a receptor for brassinolide (BL), the most active brassinosteroid. BRI1 is a leucine-rich-repeat receptor serine/threonine kinase, which belongs to a large class of kinases in Arabidopsis and other plant species. Previous studies have shown that the BRI1 kinase domian is essential for transmitting BL signals. However, the molecular and biochemical mechanisms through which BRI1 activation is regulated by BL binding are poorly understood. In this study, we have approached this problem by expressing various deletion and point mutants of BRI1 in BR biosynthetic and signaling mutant backgrounds. The detailed phenotypic analysis of stable transgenic lines under different light conditions, different concentrations of BL biosynthetic inhibitor, Brassinazole, lead to the discovery that two new motifs in BRI1 play important roles in BRI1 activation. The functions of the two motifs in regulating BRI1 kinase activation and its downstream signaling were further characterized by biochemical analysis of downstream components in transgenic lines, *in vitro* kinase assays of bacterial expressed proteins, and *in vivo* phosphorylation assays of BRI1. Furthermore, the specific residues in the two motifs, which may be directly involved in BRI1 activation, were evaluated by phenotypic characterizations of the transgenic plants that express BRI1 with various mutations in the two motifs, and the *in vitro* kinase assays of recombinant proteins from *E. coli*. Based on these findings, the activation models of this transmembrane receptor kinase will be proposed and discussed.

399 CCAAT/-binding proteins: a signaling component of the BLF-system

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Excitation of the Blue Low Fluence (BLF)-System in etiolated seedlings induces the transcription of a number of nuclear and plastid coded genes, including specific members of the Lhcb1 family; *PsLhcb1*4* and *AtLhcb1*3*. The transcriptional response is immediate and occurs in the absence of protein synthesis. Transgenic analysis of the promoter region for genes regulated by the BLF-System indicates that a perfect CCAAT box with the trinucleotide ACT immediately upstream is, in addition to the TATA box, both necessary and sufficient to allow for BLF-system induced transcription. Mobility shift analysis reveals that the BLF-system affects a DNA-binding activity recognizing this ACT/CCAAT box domain. Excitation of the BLF- system is known to activate a heterotrimeric GTP-binding protein. The use of insertion mutants, antisense lines and pharmacological agents confirm the direct role of AtGPA1, the Arabidopsis G protein alpha subunit, in BLF-system induced transcription. Yeast two-hybrid and *in vitro* binding experiments indicate that AtPirin1 is a GPA1-interactive protein and may therefore act as a signaling partner / GPA1 effector in the BLF-system. Pirin was initially identified in mammalian systems as a CCAAT-binding protein. Based on the above information it seems likely that a CCAAT-binding protein may be critical to the BLF-system induced transcription. To test this hypothesis we have obtained Arabidopsis T-DNA insertion lines for known and putative CCAAT-binding protein A, B and C subunits. When compared with wild-type seedlings, we observed a number of CCAAT-binding protein subunits, including *lec1*, fail to respond to blue light. Our data indicates that the same GPA1, pirin and CCAAT-binding protein signaling pathway may also be directly linked to sensitivity to abscisic acid during germination.

400 Cytokinin-deficient transgenic plants reveal regulatory developmental and physiological functions of the hormone

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We have engineered transgenic tobacco and Arabidopsis plants with a reduced cytokinin content in order to study for which process cytokinins are rate-limiting and possibly regulatory molecules. Plants expressing any one of six cytokinin oxidase/dehydrogenase (*AtCKX*) gene family members showed distinct developmental alterations of the shoot and root. Shoots of *AtCKX* transgenics plants had a smaller apical meristem, a slowed formation of leaf primordia, a reduced number of leaf cells, a reduced vasculature and flowered later. Visual leaf senescence did not occur earlier in *AtCKX* overexpressers suggesting that lack of cytokinins is not a physiological signal that triggers the onset of senescence. Root growth was enhanced in the *AtCKX* expressers, owing to a more rapid elongation of the primary root and lateral roots, an increased formation of lateral roots and an increased number of adventitious roots. The root meristems were larger and contained more dividing cells than in wild type plants indicating that cytokinins regulate the numbers of divisions of cells before they leave the meristem. *Promoter::GUS* fusion genes indicated differential regulation of gene family members and CKX::GFP fusion proteins were localized to different subcellular compartments. In summary, the results proved that cytokinins are a limiting factor for cell division activity in the shoot meristem, are required for leaf cell formation and have a negative regulatory function in the root. These plants are being used to study the influence of cytokinins on primary metabolism. Regulation of the local endogenous cytokinin content by the *CKX* genes is a promising tool to regulate plant organ growth and alter biomass distribution.

401 Arabidopsis Bzip genes contain a conserved uORF in their 5 UTR essential for sucrose induced repression of translation

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Plants show sugar-regulated changes in their gene expression. Different functions of hexoses and the disaccharide sucrose have been proposed in plant metabolism. Not surprising plants can distinguish between sucrose and hexoses. Investigation of the *AtbZip11* gene revealed a sucrose-induced repression of its translation. The translational control depends on the unusual long 5‘UTR. Deletion of this 5‘UTR abolishes the sucrose control (Rook et al., 1998). A conserved upstream open reading frame (uORF) is encoded in this UTR, which is homologous to other bZIP-genes in Arabidopsis and other plant species as well. The repression-system works independent of the *AtbZip11*-promoter. It is active in the shoot tissue, but absent from roots. Destroying the start-codon of the conserved uORF leads to a loss of the translational repression. Further analysis of the 5‘UTR will help to elucidate the repression-mechanism. The translation of other bZIP-genes from Arabidopsis encoding homologues of the conserved uORF is regulated by sucrose as well. The expression of the conserved uORF will be investigated by using in vitro translation assays and a uORF-peptide specific antibody. Moreover mutants with a loss of the sucrose repression are identified. Such mutants will help to understand the sensing mechanism that enables translational control.

402 Comparative expression profiling of Arabidopsis over-expressing Dof-TFs

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The aim of this project is the functional analysis of Arabidopsis transcription factors (TFs). Transgenic plants expressing altered levels of individual TFs are generated and analysed with regards to their phenotype and by comparative RNA expression profiling using the Affymetrix chip technology. We have been able to generate and screen transgenic lines overexpressing 135 different TFs belonging to TF families such as DOFs, AP2/ERFs, NACs, SCARECROWs, and others. Analysis revealed a broad range of visible phenotypes concerning overall plant size, body plan, leaf, flower and silique morphology. Metabolite analysis using GC-MS is used in selected cases to further substantiate findings from transcriptome studies. Transcriptome analysis provides a means of finding causal relationships between enhanced transcription factor activity and the phenotypic alterations observed. Together with computational investigation potential target genes of TFs can be identified, thereby providing a better insight into how individual TFs exert their function in the tissue, organ and whole-plant context. The biological role of the plant-specific DOF-type TFs has only been partially studied up to date. We performed transcriptome analysis with transgenic Arabidopsis lines overexpressing eight different DOF factors. Hybridisation data obtained from Affymetrix Gene Chip readouts were scored and evaluated using various software tools. Transgenic lines displayed unique, but to some extent also overlapping gene responses on the global level. For example one line is characterized by higher expression of genes that are related to biotic stress. Hormone regulated genes are elevated in another line, while in a third line many signalling related genes are expressed differentially as compared to wild type. In addition there is a line that displayed upregulated expression of genes related to oxidative stress and these plants are indeed more tolerant to oxidative stress than wild type plants.

403 Control of *in vitro* organogenesis by cyclin-dependent kinase activities in plants

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Organogenesis occurs in various plants tissue cultures in response to exogenously added phytohormones, mainly auxin and cytokinin. However, the molecular mechanisms that govern the developmental fate of cells during organogenesis remain poorly understood. The major regulators of eukaryotic cell cycle are cyclin-dependent kinases (CDKs). Activity of CDKs is regulated by phosphorylation as well as by association with cyclins or inhibitors. CDK-activating kinase (CAK) phosphorylates CDKs at a conserved threonine residue on the T-loop region and activates their enzyme activities. Previously, we showed that rice R2 has CDK kinase activities (1). Here, to manipulate CDK activities, we overexpressed *R2* cDNA in tobacco leaf explants by glucocorticoid-mediated transcriptional induction system. Transient expression of R2 during 7 days of culture triggered callus formation in the absence of exogenous cytokinin. This phenotype was enhanced by co-expression of rice cyclin H, which associates with and activates R2 (2), and suppressed by treatment with roscovitine, a CDK inhibitor. On the other hand, R2 expression at a later stage no longer prevent from differentiation into roots, suggesting the existence of a restricted point for sensing CDK activities that control differentiation fate of cells during organogenesis. Taken together with theses results, we propose that CDK activity is the major determinant of cell differentiation to accomplish proper development of organs.

(1); Yamaguchi *et al.*, (1998) Plant J. vol.16, 613-619
(2); Yamaguchi *et al.*, (2000) Plant J. vol. 24, 11-24

404 Molecular Cloning and Characterization of Two Abscisic Acid-Specific Glucos

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The phytohormone abscisic acid (ABA) regulates plant growth, seed maturation, dormancy and adaptive responses of plants to imposed environmental stresses. In these phenomena, ABA induces or regulates corresponding gene expression in biochemical and physiological processes. The catabolism of ABA, and its resulting inactivation, take place via two main pathways: oxidation and conjugation. Both cause ABA catabolism to increase and to accumulate phaseic acid (PA) and ABA-glucose ester (ABA-GE). The enzyme that converts ABA to PA, ABA 8'-hydroxylase, is upregulated by ABA. We isolated an ABA-inducible GTase gene from UDP-GTase homologs obtained from bean (*Phaseolus vulgaris* L.) and Arabidopsis. The deduced amino acid sequences of PvABAGT and AtUGT73B3 showed 30% to 91% identity with the known UDP-GTase homologs. Heterologous expression of the *PvABAGT* gene in *Escherichia coli* showed that the recombinant enzyme has glucosylation activity in an ABA-specific manner. When detached bean leaves were water-stressed, ABA accumulation was preceded by an increase in the *PvABAGT* mRNA level. In addition, rehydration of stressed leaves caused a rapid increase in the *PvABAGT* mRNA level. Northern blot analysis of the tissue-specific expression of *PvABAGT* in bean showed a high level of expression in the mature flowers, mature leaves, and in roots. *PvABAGT* transcript levels at different developmental stages of the pod, seed coat, and embryo were analyzed by northern blot analysis. The results indicate that the *PvABAGT* transcript is abundant in the seed coat with relatively low levels of expression observed in the pod and embryo. We conclude that expression of an ABA-specific GTase is regulated by developmental stage and environmental stress. The functional expression of PvABAGT and AtUGT73B3 in over-expressing lines and knock-out lines is in progress.

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405 The novel protein, ASB3, is required for actin cytoskeleton organization during Arabidopsis trichome development

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To probe into the mechanism of plant cell shape formation, we selected the Arabidopsis trichome, a large single cell, as a model. *ASB3* mutants were isolated from Arabidopsis populations mutagenized by fast neutrons and T-DNA insertions. In *asb3* mutants, trichomes show changes in branch position, branch length, and trichome size. Although the size of *asb3* trichomes is smaller than wildtype, its nuclear DNA content level has no alteration compared to wildtype. However, the actin cytoskeleton is disorganized in *asb3* mutants. In addition, we found the *asb3* trichomes are severely stunted when they are treated with the actin-stabilizing drug, jasplakinolide, whereas wildtype trichome development is unaffected. These results suggest that ASB3 is an actin-binding protein required for actin turnover during trichome development. We used a map-based approach to isolate the *ASB3* gene. The *ASB3* gene encodes a novel, plant-specific protein, which is located in cytoplasm.

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406 Xylem, phloem and cambium gene expression profiles as defined by tissue dissection, microarrays and tissue-specific markers

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Plant vascular tissues (xylem and phloem) support the plant body and are responsible for the transport of water, mineral solutes, nutrients and signaling molecules. The vascular cambium is the lateral meristem that produces the secondary xylem and phloem. The mechanisms that control vascular tissue differentiation remain largely unknown. To identify genes that may play a role in the differentiation and functions of plant vascular tissues, we isolated xylem, phloem, and non-vascular tissues from Arabidopsis root-hypocotyl segments and performed genome-wide comparative analysis of tissue-specific transcripts using the GeneChip Arabidopsis ATH1 genome array. Expression patterns for previously characterized xylem-, phloem- and non-vascular-specific genes exhibited the expected tissue-specific expression patterns, thereby validating the tissue dissection approach used for this study. Well-documented markers for vascular cambium activity have not been reported. Nevertheless, using markers for apical meristem and other proliferative tissues, it was determined that the majority of proliferative activity, i.e., putative lateral meristem activity, partitioned with the phloem sample. The three tissue-specific gene clusters defined by the previously established markers each contained approximately 190 genes, of which approximately 60 were classified as coding for proteins of unknown function. Considering the remaining 130 genes, the following general characteristics of the isolated tissues were noted. Xylem tissue is specialized for cell wall biosynthesis and lignification, water transport, proteolysis and the transport and catabolism of amino acids. Phloem tissue is specialized for redox mediation, signal transduction, hormone metabolism, sucrose, ion and amino acid transport, proteolysis, secondary metabolism and meristematic activity. Non-vascular tissue is specialized for stress response and defense, wax biosynthesis and nitrate uptake and assimilation. In summary, we have identified gene clusters that consist of well-known and novel genes that are likely to be required for xylem and phloem differentiation and function.

407 A chemical genetics approach to auxin signaling in Arabidopsis

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The plant hormone auxin has been intensely studied using molecular genetic and biochemical approaches, yet the mechanisms by which auxin exerts its biological effects remain poorly understood. Here we report the use of a chemical genetics approach to elucidate auxin signal transduction mechanisms in Arabidopsis. We screened for compounds that suppress or alter a particular auxin-related phenotype, and identified sirtinol, a small molecule that causes constitutive activation of many known auxin-inducible genes, leading to auxin-related developmental phenotypes. Sirtinol strongly inhibits primary root growth while stimulating adventitious root development. Moreover, sirtinol-treated plants develop cup-shaped true leaves. In an attempt to isolate sirtinol targets and downstream effectors, we identified an Arabidopsis mutant, sir1, which is resistant to sirtinol treatment but sensitive to auxin-treatment. sir1 has few lateral roots when grown in the absence of sirtinol. This work provides strong evidence that chemical genetics can be efficiently used to dissect processes implicated in Arabidopsis growth and development.

408 Functional specificity of PP2A regulatory A subunits in Arabidopsis

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Reversible protein phosphorylation mediated by protein phosphatases and protein kinases is a key regulatory mechanism governing biological processs such as the cell cycle, metabolism, transmembrane signaling and gene regulation. Our studies focus on the serine/threonine phosphatase PP2A, which consists of three subunits: regulatory subunits A and B, and catalytic subunit C. In Arabidopsis the regulatory A subunit is encoded by three genes. The *RCN1* gene (At1g25490) encodes the α isoform, while At3g25800 and At1g13320 encode the β and γ isoforms, respectively. The *rcn1* mutant exhibits abnormalities including defects in polar auxin transport, differential cell elongation and ABA response, suggesting specific roles for the α isoform in these processes. In order to characterize the roles of the two other regulatory A subunit gene family members, we have isolated new mutants carrying T-DNA insertions in At3g25800 and At1g13320. Immunoblotting experiments show that each mutant lacks one of the A subunit proteins, suggesting that the mutations are knock-outs. Both new mutants exhibit slight phenotypic differences from the wild-type Columbia parent, but neither mutant phenotype resembles that of the *rcn1* mutant. Construction of double mutant lines results in severe phenotypes including abnormal embryogenesis, severe dwarfing, and sterility. Together these data suggest that the regulatory A subunit isoforms fulfill partially overlapping biological roles, and that maintenance of PP2A regulation is crucial for normal plant growth.

409 Spatial and temporal expression of KUP/HAK/KT genes in Arabidopsis

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Potassium (K^+) is the most abundant cation in plants and large quantities are required for plant growth. To ensure an adequate supply of K^+, plants have multiple mechanisms for uptake and re-translocation. There are five families encoding K^+ transporters in Arabidopsis. One large family of transporters contains 13 genes. These genes have been named *KUP/HAK/KT* but relatively little is known about their function. Our aim was to determine the spatial and temporal expression patterns of each *KUP/HAK/KT* gene. Knowledge of where each of the genes in the family are expressed is essential for understanding the role of these transporters in controlling potassium homeostasis (1-4) and plant development (5, 6). To determine where and when the *KUP/HAK/KTs* are expressed, we used real time PCR with SYBR green. Plants were grown in hydroponic solutions. The growth conditions included K^+ deprivation for one and six days, K^+ deprivation then resupply of K^+, and excess K^+ and Na^+. We analyzed *KUP/HAK/KT* gene expression across a range of tissues including roots, old leaves, young leaves, developing siliques, and flowers under the different conditions. Expression patterns under the different conditions, in different tissues and in a range of cell types in the root will be presented.

1. H. Fu, S. Luan, *Plant Cell* 10, 63-73 (1998).
2. E. J. Kim, et al. *Plant Cell* 10, 51-62 (1998).
3. G. E. Santa-Maria, et al. *Plant Cell* 9, 2281-2289 (1997).
4. F. J. Quintero, M. R. Blatt, *FEBS Lett.* 415, 206-211 (1997).
5. R. P. Elumalai, P. Nagpal, J. W. Reed, *Plant Cell* 14, 119-131 (2002).
6. S. Rigas et al., *Plant Cell* 13, 139-151 (2001).

410 Methyl jasmonate-, salicylic acid- and Bion-mediated changes in *Arabidopsis thaliana* P450 gene expression in microarrays

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Methyl Jasmonate (MeJ), Salicylic Acid (SA) and Bion™ (azibenzolar-S-methyl or BTH active ingredient), which are known as chemical activators and signaling molecules, induce a wide range of plant biochemical reactions involved in the production of defense-related compounds in plant cells. They believed to regulate expression of genes involved in systemic acquired resistance (SAR) and genes coding for proteinase inhibitors, for synthesis of phytoalexin, flavone and other defense-related compounds. P450 gene specific microarray techniques have been used to explore any changes in P450 gene expression that occur with application of these three chemicals. For our studies, eight-days-old seedlings were treated either with 100 iM MeJ, 1 mM SA or 200 mg L-1 Bion for periods between 1 and 30 hours. Detailed analysis of microarray data indicate that 39 (MeJ), 29 (SA) and 44 (Bion) P450 transcripts showed induction of at least 2-fold over same stage controls lacking any chemical treatment. Also, MeJ, SA and Bion induce 15, 8 and 22 biochemical pathway marker genes that have been arrayed alongside with these P450s. In these comparisons, MeJ showed the highest range of inductions followed by Bion and SA. While these data highlight specific sets of P450 transcripts responding to these chemicals, there are also distinct overlaps in the responses of several P450 loci: MeJ and SA inducing 1 locus, MeJ and Bion inducing 10 loci, SA and Bion inducing 22 loci and all three chemicals inducing 6 loci. These data also indicate that 2 (MeJ), 20 (SA) and 14 (Bion) P450 transcripts are repressed at least 2-fold. These experiments highlight a range of P450 and biochemical pathway loci responding in concerted fashions to these chemical treatments.

411 Chitin: An elicitor of plant defense in *Arabidopsis*

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Previously it was shown that chitin oligomers, released from fungal or insect cell walls by endochitinase, induce defense and related cellular responses in many plant species. Recently we described in *Arabidopsis* the gene expression patterns responding to chitin oligomers treatment, using a microarray consisting of 2375 EST clones representing putative defense-related and regulatory genes (Ramonell et al., 2002). The results indicate that chitin elicits a specific response in *Arabidopsis* through a unique pathway distinct from the salicylic and ethylene/jasmonate pathways (Zhang et al., 2002). Our objective is the identification of components of the chitin signal transduction pathways using reverse genetics. Here, we describe the first part of the characterization, including the phenotype after infection with biotrophic and necrotrophic fungi, of different T-DNA insertion mutants affected in genes, which are up-regulated after treatment with chitin oligomers. Some of these genes are implicated in protein-protein and DNA-protein interactions of different steps of chitin response transduction pathway, others are implicated in direct defense responses against pathogens. Our results about it will be present at this poster. See the complimentary poster by our collaborators, Jinrong Wan et al.

Ramonell K, M, Zhang B, Ewing R, M, Chen Y, Xu D, Stacey G and Somerville S. Microarray analysis of chitin elicitation in *Arabidopsis thaliana*. Mol Plant Pathol. 2002 3(5): 301-311.

Zhang B, Ramonell K, Somerville S, Stacey G. Characterization of early, chitin-induced gene expression in *Arabidopsis*. Mol Plant Microbe Interact. 2002 Sep;15(9):963-70.

412 Hormonal interactions during ozone stress in Arabidopsis thaliana

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To investigate plant stress responses an *Arabidopsis thaliana* "stress microarray" was constructed. The Arabidopsis "stress microarray" has a total of 1536 elements. Of these 1076 were identified from differential screening of eight subtracted cDNA stress libraries [acute ozone, chronic ozone, salicylic acid, methyl jasmonate, virulent and avirulent bacteria (Pseudomonas syringae) and oomycetes (Peronospora parasitica)]. Another 396 ESTs were selected based on reports from other microarray studies of plant defense responses. The remaining elements were negative controls (15) and spiking controls (49). Hybridization signal intensities from the spiking control spots were used to normalize the data. The "stress microarray" was used to investigate the early responses to the air pollutant ozone. In particular we want to know the role of stress hormones (ethylene, jasmonic acid, salicylic acid and abscisic acid) and cell death regulators (RCD1) in the ozone stress response. Single mutants (*abi2, ein2, jar1*, NahG, *npr1, rcd1*), double mutants (*jar1**NahG, *ein2**NahG, *jar1*ein2*, *rcd1*ein2*, *rcd1**NahG, *rcd1*npr1*) and wild types (Ler, Col) were exposed to ozone and plants were taken for microarray analysis after 30 min, 1h, 2h, 4.5h and 8 hours. Approximately 700 of the genes were up-regulated at least 2-fold at one or more of the time points following ozone exposure in wild type Columbia. Preliminary analysis of the data indicate that the expression pattern of a number of genes is unaffected in all mutant backgrounds tested indicating that these genes were maybe regulated through other mechanisms than the characterized stress hormone signaling pathways.

413 Understanding Early Events in Phytochrome Signal Transduction

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Plants utilize light as a source of energy as well as a source of information about the environment. Light direction, intensity, duration, and wavelength are monitored in the plant by three photoreceptor systems, one of which is the red/far-red light absorbing phytochromes. Seed germination and seedling de-etiolation are two developmental programs controlled in part by the action of phytochrome A (phyA). It is known that both subcellular localization and kinase activity of phyA are light regulated. However, the connection between these processes as well as the substrate(s) for phyA are unknown. We are taking a combined proteomic- and cell biology-based approach to understand the early events in light-induced phytochrome signaling. The goal of this project is to to determine if phyA is involved in the nucleocytoplasmic partitioning of known but uncharacterized signaling components, and whether protein trafficking in general is responsive to light. Furthermore, this project aims to uncover novel early signaling components that have remained unidentified due to lethality or redundancy. These projects should offer valuable insight into the mechanisms by which phyA mediates photomorphogenesis.

414 Auxin and Shade Avoidance Response

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Plants have evolved sophisticated sensing mechanisms, operating through phytochromes, in order to perceive changes in the red:far-red ratio of the light environment and trigger morphological changes to avoid shade. Recent studies in Arabidopsis implicated ATHB-2, a homeodomain-leucine zipper transcription factor, as a regulator of shade avoidance responses downstream of phytochromes, and established a strong link between this factor and auxin signaling. On the basis of these findings, we postulated a model in which low R:FR light, via ATHB-2, induces a re-direction of auxin flux from the vasculature to external cell layers, resulting in a reduced auxin flux in the root. To support the model, we examined the auxin-responsive expression of DR5::GUS in seedlings exposed to shade light. GUS expression was significantly increased in cotyledons and hypocotyls of DR5::GUS seedlings exposed to 4 h FR-rich-light illumination. Furthermore, cross-sections of hypocotyls revealed the GUS staining in all the outer cell layers. To directly correlate the GUS signal with auxin levels, we measured the auxin content in the aerial part of seedlings exposed to shade light by mass spectrometry. We observed significantly elevated free IAA levels ($P < 0.05$) in the aerial part of seedlings exposed to 4 h FR-rich-light illumination, as compared to controls. Thus, direct measurement of free IAA content confirmed the DR5-based observations, supporting the notion that shade light provokes changes in auxin levels. Consistent with these findings, Northern blot analysis revealed that the expression of many auxin-regulated genes, such as IAA, SAUR and GH3 genes is induced by FR-rich light. A kinetic analysis of IAA gene expression in wild-type plants and mutants impaired in auxin response exposed to FR-rich light is presently in progress.

*These authors contributed equally to this work.

415 CNGC2, a cyclic nucleotide-gated ion channel, is crucial for plant development and adaptation to calcium stress

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Cyclic nucleotide-gated channels (CNGCs) facilitate the conduction of various cations, including Ca^{2+}, Na^{+} and K^{+}. These channels are important for sensory transduction in animal systems (1) but their functions in plants are much less understood. *Arabidopsis CNGC2* (also called *DND1*, (2)) can form an ion channel that allows Ca^{2+} and K^{+} permeation but interestingly, not Na^{+} current (3, 4). Genetic studies showed that its disruption results in a near-complete loss of the hypersensitive response (HR). Moreover, *cngc2* mutant plants are dwarf that also exhibit constitutively heightened defense against various pathogens (2, 5). To better understand how ion permeation through CNGC2 affects HR, disease resistance and other developmental processes, we characterized the sensitivity of *cngc2* mutants to various ions in the growth medium. We showed that *cngc2* plants are specifically hypersensitive to Ca^{2+}. This results in stunted vegetative growth and reduced reproductive fitness (6). To our knowledge, this is the first report of a significant mutant phenotype caused by challenging plants with physiologically relevant extracellular [Ca^{2+}]. Our data support the hypothesis that CNGC2 is critical for plant development, particularly in physiological calcified conditions.

Our results also suggest that CNGC2 affects numerous developmental processes, including vegetative and reproductive growth, programmed cell death, and adaptive responses to biotic and abiotic stimuli. We are taking a multi-disciplinary approach to studying the CNGC2 protein, and its role in Ca^{2+} signaling, gene transcription and protein expression. This work will enhance our understanding of the relationships between CNGC2, Ca^{2+} signaling, and various important physiological processes.

1 Kaupp and Seifert (2002) Physiol Rev 82:769-824.
2 Yu et al., (1998) Proc Natl Acad Sci USA 95: 7819-7824.
3 Leng et al. (1999) Plant Physiol 121: 753-761
4 Leng et al. (2002) Plant Physiol 128: 400-410
5 Clough et al., (2000) Proc Natl Acad Sci USA 97:9323-9328.
6 Chan et al., (2003) Plant Physiol in press.

416 The ascorbate-deficient Arabidopsis mutant *vtc1* has altered responses to both high light and pathogens.

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When exposed to high light, xanthophyll cycle activity is significantly lower in the ascorbate-deficient *vtc1* mutant than in wild type. This is accompanied by decreased energy-dependent quenching, resulting in diminished non-photochemical quenching of chlorophyll a fluorescence in *vtc1*. In contrast, photoinhibitory quenching is the same in both genotypes after high-light exposure. This is consistent with the decrease in potential photosystem II activity measured as ratio of maximum to variable chlorophyll a fluorescence (FV/FM) that declined to the same extent in wild type and *vtc1*. Moreover, potential photosystem I activity which was determined by P700 absorbance changes at 810 nm is similarly affected in both genotypes under high light. Elevation of the endogenous ascorbate content by feeding with the direct precursor of ascorbate, L-galactono-1,4-lactone, restored xanthophyll cycle activity and non-photochemical quenching in *vtc1*, but the degree of photoinhibition was neither diminished in wild type nor in *vtc1*. Given these results, we conclude that the ascorbate deficiency in *vtc1* does not lead to pronounced oxidative stress generated by high light in the chloroplast. Infection of wild-type and *vtc1* leaves with either virulent *Pseudomonas syringae* or *Peronospora parasitica* resulted in largely reduced bacterial and hyphae growth, respectively, in *vtc1* compared to wild type, indicating that *vtc1* is more resistant to these, otherwise virulent, pathogens. Moreover, we observed elevated induction of pathogenesis-related proteins PR-1 and PR-5 upon challenge with a virulent strain of *Pseudomonas syringae*. We surmise that this elevated induction of PR proteins in *vtc1* may contribute to pathogen resistance.

417 Coordination of Development and Metabolism by the Circadian Clock

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Circadian clocks regulate many essential cellular processes in most organisms studied, controlling major metabolic pathways and developmental decisions. Our understanding of how the clock controls different output pathways to temporally-regulate physiological and developmental processes is incomplete. We are studying the strategies used by the clock to differentially control subsets of output genes and pathways.

A previous circadian microarray experiment to identify circadian-regulated genes in Arabidopsis showed that the transcript levels of three members of the basic Helix-Loop-Helix family of transcription factors have been found to be circadian-regulated. These Circadian-Regulated bHLHs (CRBs) belong to a well-characterized family of DNA-binding proteins that have been implicated in diverse developmental pathways as important transcriptional regulatory components and are predicted to bind to the G-box motif found in many light-regulated promoters in plants. bHLH transcription factors are also well-represented in the circadian systems of other organisms, from Drosophila to humans. The circadian clock in mice, for example, has four bHLHs involved in a transcription-translation feedback loop responsible for generating these rhythms.

The CRBs identified in Arabidopsis are expressed at different phases throughout the day and appear to be involved in the regulation of distinct physiological pathways. To study how the clock uses such transcription factors to control specific pathways, we have made use of Affymetrix?s full genome Arabidopsis GeneChips to evaluate the effect of CRB-overexpression at different times of day.

The previous microarray analysis also demonstrated that 453 of the ~8,200 distinct genes represented on the original Affymetrix Arabidopsis GeneChip are circadian-regulated. If these proportions are representative for the entire genome, then there are over 1,000 circadian-regulated genes in Arabidopsis that have yet to be identified as being rhythmically expressed. Therefore, we are also analyzing the circadian expression of the entire Arabidopsis genome using the full genome GeneChips. Results will be presented on the different pathways that exhibit clock regulation, the roles of the CRBs, and how the clock coordinates plant metabolism and development.

418 Genetic Mechanisms Regulating Root System Architecture

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The architecture of a plant's root system is critical to its survival. We have shown that *Arabidopsis thaliana* var. Columbia (wildtype) root systems can develop distinct morphologies in response to their environment: when water is plentiful, seedlings develop highly branched root systems, whereas when seedlings are grown under mild drought conditions (osmotic stress), completely unbranched root systems are formed. Although the complete mechanism of this developmental difference is unknown, we have found that it is modulated by the plant hormones abscisic acid (ABA) and auxin. We have isolated a number of mutants in *l*ateral *r*oot *d*evelopment (*lrd*) in a screen for plants unable to repress lateral root proliferation in response to osmotic stress. In addition to this phenotype, *lrd1* and *lrd2* are sensitive to salt in germination assays, and are drought tolerant. Because these two traits are both ABA-dependent, this suggests that the LRD genes may play a role in mediating ABA responses. Although both mutants were identified in the same screen and have some similar phenotypes, further analysis has shown that these two mutations cause distinct disruptions in regulation of root system architecture. *lrd1* makes more lateral roots than wildtype under all conditions, but the number of lateral roots is nonetheless responsive to osmotic stress. In contrast, *lrd2* makes more lateral roots than wildtype under all conditions and the number of lateral roots produced is similar in both water-plentiful or osmotic stress conditions. Hence, these mutations define two separable pathways that determine root system architecture: one pathway that is osmotic-independent, and which involves both *LRD1* and *LRD2*; and a second pathway that is responsive to osmotic stress, and involves only *LRD2*. This suggests that there may be at least two ways in which the development of a plant's root system can be regulated: either by genetic loci which determine the intrinsic morphology of the root system, or by loci that determine the ability of the plant to perceive and respond to its environment. Progress in cloning and molecular characterization of LRD1 and LRD2 will be discussed.

419 *frd4*: a Mutant with Altered Iron Deficiency Responses

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Iron is essential for many cellular processes in plants such as photosynthesis and respiration. However, the over accumulation of iron can have detrimental effects due to its ability to catalyse the formation of damaging hydroxyl radicals. Plants therefore possess carefully regulated mechanisms to acquire iron from the soil. Part of one such mechanism, the so-called Strategy I response found in dicots and non-graminacious monocots, involves the activation of a root Fe(III) chelate reductase under iron deficient conditions. This reductase, encoded by the *FRO2* gene, functions to convert iron from its insoluble Fe(III), or ferric, form to its more soluble Fe(II), or ferrous, form. The Arabidopsis mutant *frd4* (ferric reductase deficient 4) was isolated in a screen looking for plants that failed to upregulate their Fe(III) reductase activity under low iron conditions. Interestingly, *frd4* expresses *FRO2* normally under iron deficiency, suggesting that FRD4 acts on FRO2 post-transcriptionally. Further, the *frd4* mutation is pleiotropic: *frd4* plants are smaller and more chlorotic than *frd1* mutants which contain mutations in *FRO2* and thus also lack iron-deficiency inducible root Fe(III) chelate reductase activity. The *frd4* mutation must therefore affect other proteins besides FRO2. Proposed functions for FRD4 include (1) the stable accumulation of FRO2 protein, (2) the correct localisation of FRO2 protein, (3) the supply of cofactors to FRO2, and (4) the activation of FRO2. Map-based cloning has narrowed the *frd4* mutation to a 137kb region, containing 48 predicted open reading frames, at the bottom of chromosome 2. In addition to the final cloning of *frd4*, future plans involve the additional characterisation of the mutant and of the wild type FRD4 gene product.

420 A bZIP transcription factor activated by glycine betaine and required for chilling tolerance in Arabidopsis

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Glycine betaine (GB) is known to confer stress tolerance in several plants. We have been interested in the hypothesis that at least part of GB's effect can be attributed to gene activation. Pursuing this idea with Arabidopsis microarrays, we identified >350 genes whose expression is strongly activated by GB treatments. Among genes activated are several for cell wall & carbohydrate metabolism, integral cell wall proteins, lipid metabolism, and anti-oxidant related enzymes as well as regulatory proteins. One of these regulatory proteins is a bZIP transcription factor. Northerns confirm that its expression is activated >4X by glycine betaine treatment. A knockout Arabidopsis for this gene has altered stress tolerance compared to controls with respect to germination on high salt as well as electrolyte leakage/superoxide production under chilling conditions. Thus, these results demonstrate that GB can activate gene expression protecting plants from stress.

421 Heavy Metal Transport ATPases in Arabidopsis

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P_{1B}-type ATPases transport heavy metals from the cytoplasm into organelles and the extracellular milieu. Genomic information from A. thaliana shows the presence of eight different genes encoding for P1B-type ATPases: RAN1, PAA1, HMA1-6. Analysis of conserved amino acids in the transmembrane fragments flanking the large cytoplasmic domain suggests that RAN1, PAA1, HMA5 and HMA6 are Cu^{+}-ATPases, HMA1 is a Co^{2+}-ATPase and HMA2, HMA3 and HMA4 are Zn^{2+}-ATPases. Among these enzymes, only RAN1 has been partially characterized. Toward understanding the roles of these enzymes in micronutrient mineral metabolism, we have cloned the seven other ATPases (PAA1, HMA1-6). We have determined the level of their transcripts in different organs and in seedlings exposed to different metals. Results of these studies suggest that while some have specific tissue distribution (HMA5 and HMA6 for instance) others appear to be ubiquitous. In both cases, they are regulated in response to specific metal exposure. Initial screening of available seed stocks carrying T-DNA insertions in the genes of interest suggests that disruption of these genes is not lethal.

422 The role of PRR7 in the Arabidopsis circadian clock

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In *Arabidopsis* a negative feedback loop involving the genes *LHY*/*CCA1* and *TOC1* have been proposed to participate in the central oscillator of the circadian clock (Alabadi et al., 2001). *TOC1* is a paralogue to a family of circadian regulated pseudo response regulator (*PRR*) genes (Matsushika et al., 2000). In this work we characterized plants carrying a null mutation of *PRR7*. Under constant conditions this mutation increases the period of leaf movement, and the expression of *CCR2::luc* and *CCA1* by about one hour. In addition, *prr7* mutants have longer hypocotyls in light/dark cycles, and constant red and far red light. They flower slightly late under long day and early under short days. Furthermore, this mutation leads to changes in expression level of circadian regulated genes such as *CCA1* and *PRR9*. From these results we conclude that *PRR7* plays a role in the regulation of the *Arabidopsis* circadian clock.

Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Mas P, and Kay SA (2001). Reciprocal regulation between *TOC1* and *LHY*/*CCA1* within the *Arabidopsis* circadian clock. Science 293, 880-883. Matsushika A, Makino S, Kojima M, and Mizuno T (2000). Circadian waves of expression of the *APRR1*/*TOC1* family of pseudo response regulators in *Arabidopsis thaliana*: Insight into the plant circadian clock. Plant Cell Physiol. 41(9),1002-1012.

423 Effect of phenobarbital, atrazine, and cold on Arabidopsis P450s and biochemical pathway marker transcripts

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Atrazine, a herbicide, is known to inhibit electron transport by binding to photosynthesis II reaction centre protein, D1, in thylakoid membranes. Phenobarbital, a marker for environmental pollution, is known to induce P450-dependent enzyme activities in plants. To define the range of P450 loci induced or repressed by the these xenobiotics, transcript profiles in 8-day-old Arabidopsis seedlings treated for 6 and 24 hours with either 500 uM phenobarbital or 100 uM atrazine were defined in P450 gene specific microarray. Analysis of these arrays relative to same stage untreated control seedlings has indicated that 6 hrs exposure to phenobarbital induced 9 P450 genes and repressed 14 pathway markers and 24 hrs exposure to phenobarbital did not affect expression of any microarray elements. In contrast, 24 hrs exposure to 100 uM atrazine treatment induced 3 P450 and 2 pathway marker genes. None of the microarray elements showed repression by 6 hours or 24 hours atrazine treatment. Treatment on 7-day-old seedlings at 4C induced 6 P450 and 4 pathway marker genes and repressed 5 P450 and 3 pathway marker genes after 3 hrs cold treatment. Treatment at 4C for 27 hrs induced 21 P450 and 17 pathway marker genes and repressed 35 P450 and 17 marker genes. Although treatments are not necessarily comparable, atrazine was the least effective treatment modulating P450 transcripts.

424 Exogenous *all-trans* Retinal Rescues Light-Mediated Growth Defects of a Putative Carotenoid Cleavage Mutant

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The chromophore for opsin-based light sensing is synthesized from the symmetrical cleavage of B-carotene. In the animal eye, this is catalyzed by the enzyme RPE65. *Arabidopsis thaliana* contains a family of at least 8 putative 9-*cis*-epoxycarotenoid dioxygenases, all which share some homology with animal *RPE65*. One specific member, *CCD8*, has particularly strong local homology to the animal retinal-generating enzyme required for opsin-based light sensing. Retinal-based photoreceptors have been identified in animal, bacterial, fungal and algal species, but have not been identified in plants. If such a light sensor were to exist in plants, it would likely be dependent upon the activity of CCD8 to generate retinal for use as a chromophore. To test if this enzyme has a role in plant light responses, reverse-genetic mutants were identified and assayed for photomorphogenic phenotypes. Mutant seedlings exhibit light-dependent defects in hook opening and hypocotyl/root elongation. Defects in root elongation are particularly conspicuous and are affected by light quality and quantity. The root phenotypes are especially apparent after seedlings are positioned perpendicular to gravity. Although the root tip orients correctly, the root fails to elongate, indicating the phenotype arises from a defect in elongation, not gravity sensing. Normal root elongation can be restored in the mutant by addition of micromolar concentrations of *all-trans* retinal to the growth media. These findings support the following possible conclusions: 1. Plants may contain a retinal-based photoreceptor that directs elongation of specific organs, or 2. Plants may use retinoids, or a product derived from retinoid metabolism, to regulate aspects of photomorphogenic development.

425 Blue-light Induced Increases in Cytosolic Calcium are Required for Primary Inhibition of Hypocotyl Elongation, not Phototropism

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The phototropin 1 photoreceptor (phot1) is responsible for hypocotyl phototropism induced by unilateral blue light and for the onset of growth inhibition induced by symmetric high-irradiance blue light. The photobiology of phot1-mediated phototropism has been characterized extensively already, but not the primary phot1 phase of growth inhibition which precedes the influence of the cryptochromes when etiolated seedlings are irradiated with blue light. Here we show that a pulse of blue light delivering between 1 and 1000 μmol m^{-2} of photons caused a transient inhibition of hypocotyl elongation that peaked 15 min after the pulse. The pulse-induced inhibition depended entirely on phot1 without contributions from other blue-light receptors. Like phototropism, the response obeyed the Bunsen-Roscoe law of reciprocity and therefore could be used to assay the transduction chain linking phot1 to the control of elongation growth. The earliest transduction step initiated by phot1 is stimulation of its own intrinsic kinase activity. This step is followed within a few seconds by activation of plasma-membrane Ca^{2+} channels, resulting in a transient rise in cytoplasmic Ca^{2+} concentration over a time course of approximately 40 s. The goal of the present work was to determine whether the rise in Ca^{2+} was a signal transduction event on the pathway leading to either phototropism, growth inhibition, or both of these phot1 responses. Treatment of seedlings with a Ca^{2+} chelator prevented the rise in cytoplasmic Ca^{2+} and blocked phot1-mediated growth inhibition. However, the same chelator treatment did not impair phot1-mediated phototropism. Thus, the early, transient rise in cytoplasmic Ca^{2+} is an important intermediary process in at least one but not all signaling pathways initiated by phot1.

426 Motabolite Regulation of Amino Acid Permease I (AAP1)

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In higher plants, amino acids are the currency of nitrogen exchange between the sites of primary assimilation and the import-dependent tissues. The partitioning of amino acids in this resource allocation process requires the activity of several classes of amino acid transporters in the plasma membrane. The transcript of AAP1, a proton-amino acid symporter, in mature leaf tissue is regulated by nutrient status and environmental cues. After 7 days nitrogen starvation, AAP1 message is highly induced after feeding 30 min in 25 mM $NO3^-$ or 10 mM $NH4^+$ or 5 mM amino acids such as glutamine, glutamate and asparagine. Nitrogen mediated AAP1 message changes may be due to multiple signals. Inhibitors of nitrate reductase, glutamine synthetase, and aminotransferases do not block induction by $NO3^-$, $NH4^+$ and asparagine, respectively, while glutamate synthase inhibitors decrease the induction from glutamine, suggesting the importance of glutamine-glutamate cycle in nitrogen signaling. AAP1 is also induced in dark-adapted plants after 3 hours of illumination. Light dependent changes in expression may be mediated by a specific photoreceptor or by photosynthesis-dependent increases in leaf sugar content. Both 1% sucrose or glucose feeding induces AAP1 message in dark- adapted plants, suggesting light induction might be an indirect effect of sugar-signaling. However, we can not rule out a role for a photoreceptor in regulation of AAP1 message, because far red light illumination decreases the sucrose-dependent induction. AAP1 promoter has been used to make promoter:reporter constructs with GUS and luciferase. The nitrate mediated expression of reporter gene driven by AAP1 promoter is same as that of native gene. Reporter gene expression has also been identified in flower and developing seeds, and AAP1 knock out plants showed delayed inflorescence as well as lower productivity, suggsting function of AAP1 in mobilization of nutrient in developing seeds. Current efforts in the lab include parallel experiments using expression profiling to identify transcription factors associated with changes in AAP1 expression, and developing genetic tools for dissecting this response pathway.

427 Role of HFR1 in phytochrome signalling

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HFR1 (long hypocotyl in far-red) is a bHLH protein involved in phytochrome A signaling. Arabidopsis seedlings deficient in HFR1 have longer hypocotyls and smaller cotyledons than normal when grown in continuous far-red light. An analysis of the *hfr1* mutant visible phenotype at various time points was performed. This analysis showed a visible *hfr1* phenotype as early as 24h after the onset of far-red light. In order to identify HFR1 regulated genes, we are comparing expression of phytochrome A regulated genes in *hfr1* mutants and wild-type within the first 24h of far-red light. Preliminary results may indicate the involvement of HFR1 in the suppression of shade avoidance. Since shade avoidance is thought to be mediated in part by auxin transport, the potential involvement of auxin in the *hfr1* phenotype is being explored by use of the auxin polar transport inhibitor NPA.

428 *Cis*- and *trans*-acting factors controlling clock-regulated gene expression

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Like many other organisms, plants possess internal biological clocks that help them coordinate internal events with the external environment. These circadian clocks allow plants to anticipate the environmental changes that occur every 24 hours, most notably the rising and setting of the sun. Circadian rhythms provide organisms with an adaptive advantage, presumably by allowing them to schedule physiological events so that they occur at the most advantageous times. In many organisms, a transcriptional/translational feedback loop lies at the heart of the circadian oscillator. This central clock often controls output genes at the transcriptional level.

We have previously identified a motif found in the promoters of many evening-phased, clock-regulated genes and showed that this evening element (EE) is required for conferring circadian rhythmicity on a reporter gene (Harmer et al, *Science*, 2000 **290**:2110-3). The EE may also play an important role in the function of the central oscillator itself (Alabadi et al, *Science*, 2001 **293**:880-3).

In work presented here, we demonstrate that the EE is sufficient to confer evening-phased expression on a reporter gene, and characterize *trans*-acting factors that mediate this clock-regulated expression.We also identify a new promoter motif sufficient to confer dawn-phased expression on a reporter gene. Through these studies, we hope to learn how the circadian clock regulates two distinct phases of gene expression, shedding light on how the clock allows plants to time physiological events most advantageously.

429 *AFR* is a positive regulator of phytochrome A-mediated light signaling

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An insufficiently understood aspect of the circadian oscillator in *Arabidopsis thaliana* is the molecular makeup of the interface between the central clock and its input/output pathways. To identify additional components of this circuitry, we employed a functional genomics approach in which RNA interference (RNAi) was used to individually reduce the expression of a series of cycling candidate genes of unknown function, followed by screening of these lines for circadian defects. Several independent transgenic lines harboring an RNAi construct that targeted the novel gene *ATTENUATED FAR RED RESPONSE* (*AFR*) displayed a series of phenotypes consistent with impaired phytochrome A (phy A)-mediated light signaling. *afr-R* seedlings have elongated hypocotyls under low fluences of red, blue, and far-red light, in addition to reduced acute induction of light-responsive genes after far-red light treatment. *AFR* appears to act downstream of phyA, since a reduction in *AFR* expression suppresses the short hypocotyl phenotype caused by overexpression of phyA. This gene encodes a protein having a predicted F-box domain, and the full-length protein interacts with the SCF complex component *Arabidopsis* SKP1. Therefore, AFR may potentiate light signals transmitted through the phyA signaling network by targeting a repressor of this pathway for degradation by the 26S proteasome.

[1]FGH is a DOE-Energy Biosciences Research Fellow of the Life Sciences Research Foundation

430 *BON1* Regulates Cell Growth Through an *R*-like gene

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BON1 encodes a calcium-dependent phospholipid binding protein belonging to an evolutionarily conserved copine family. The Arabidopsis *bon1* mutant has a dwarf phenotype due to reduced cell size at 22?C and this dwarf phenotype is suppressed by high temperature 28?C, indicating a role of *BON1* in maintaining growth homeostasis. The *bon1* mutant also conditionally exhibits enhanced disease resistance, suggesting *BON1* being a negative regulator of defense response. We show here that the reduction in cell growth in bon1 requires an activation of a homolog of *RPP5*, an *R* gene in pathogen defense. The dwarf phenotype of *bon1* is dependent on the ecotype it resides in, and genetic analysis revealed the *MOB1* gene as the primary modifier of bon1. The *MOB1* gene is cloned as an *R*-like gene (*Col-B*, *SNC1*, and *BAL*) in the *RPP5* gene cluster. The dwarf phenotype of *bon1* is manifested in Col-0 ecotype with a functional *Col-B* gene and is absent in Ws due to a non-functional *Col-B*. The *Col-B* gene is upregulated in *bon1* and *bon1-like* mutants such as *cpr1* and *bal* at 22?C, and its expression is suppressed at 28?C correlating with a rescue of their dwarf phenotype. Thus *BON1* is a negative regulator of the *Col-B* gene that inhibits cell growth. We propose that the loss of *BON1* function perturbs cellular homeostasis and subsequently activates *Col-B*, and the activation of *Col-B* represses cell growth and induces pathogen defense responses.

431 Nuclear translocation of the photoreceptor phytochrome B is necessary for its biological function.

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The phytochromes (phy) are a family of informational photoreceptors (designated phyA to phyE in Arabidopsis) that enable plants to optimize their growth and development in constantly changing natural light conditions. Although the phy signaling mechanism is still elusive, recent discoveries have significantly changed our views about the molecular, cellular and biochemical processes involved in the signaling pathway. Importantly, although phys were previously considered to be constitutively cytoplasmically localized soluble proteins, recent studies have shown that all five phys translocate into the nucleus in response to light. However, direct evidence of the functional relevance of this nuclear translocation is still lacking. Using a glucocorticoid receptor(GR)-based fusion protein system, we provide direct evidence that nuclear translocation of phyB is necessary for its biological function.

432 The role of FKF1 in the photoperiodic control of flowering in Arabidopsis

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One of the pathways governing flowering time in Arabidopsis is a photoperiodic pathway. Arabidopsis plants flower much more rapidly in long days compared to short days. The *fkf1*(flavin-binding, kelch repeat, F box) mutant was isolated as a late flowering mutant in long days and appears to be photoperiod insensitive. The original *fkf1* mutant was obtained from fast-neutron mutagenized seed populations and contains a large 60 kb deletion encompassing FKF1. We therefore decided to obtain a T-DNA insertion line to confirm the *fkf1* mutant phenotype. Our insertion line showed a late flowering phenotype in long days, which reinforced the observation that FKF1 is involved in the photoperiodic pathway of flowering. The circadian clock controls the daily changes of accumulations of the *FKF1* transcripts. We checked whether or not FKF1 has some feedback function to a central oscillator. The rhythms of both *cab2::luc* and *ccr2::luc* reporter genes in *fkf1* were very similar to those in wild type. The overexpression of *FKF1* also did not affect *cab2::luc* rhythm. These results suggest that FKF1 does not affect the clock running, so FKF1 might mediate day length signals from the clock to downstream genes to lead into flowering. Recently, a possible molecular mechanism underlying the photoperiod measurement was reported. In this model, the coincidence of a high-level accumulation of the flowering activator CONSTANS (CO) and light leads to the accumulation of *FLOWERING LOCUS T (FT)* transcripts. Although it is still unknown how CO waveform is generated, the circadian clock also controls the accumulation of <I)CO mRNA. We examined the accumulation levels of *CO* and *FT* transcripts in both wild type and *fkf1* mutants. We observed that the *CO* and *FT* expressions were altered in the *fkf1* mutants, which might cause a late-flowering phenotype of *fkf1*. The function of FKF1 in photoperiodic measurement according to the characterization of *fkf1* mutants will be discussed.

433 Phototropins choose signal transducers, RPT2 and NPH3, according to three different blue-light responses

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Phototropin 1 (phot1) and phot2, which are blue-light receptor kinases, function in blue-light-induced hypocotyl phototropism, chloroplast relocation, and stomatal opening in *Arabidopsis thaliana*. It is interesting that different movement responses to blue light in different tissues of a plant (hypocotyl, mesophyll cells, and guard cells) are mediated through the same photoreceptors, phot1 and/or phot2. How do phot1 and phot2 transfer the blue-light signal for different blue-light-induced responses? It is possible that phot1 and/or phot2 may choose signal transducers to suit each response. Previous studies have shown that the proteins RPT2 and NPH3 transduce signals downstream of phototropins to induce the phototropic response. However, the involvement of RPT2 and NPH3 in stomatal opening and chloroplast relocation mediated by phot1 and phot2 is unknown. To examine the relationship between phototropins and signal transduction molecules, we analyzed the blue-light responses of *phot1-101*, *phot2-5*, *rpt2-1*,*nph3-101*, and their double mutants. On the basis of genetic observations, we propose three models of signaling pathways controlling blue-light-induced movements, hypocotyl phototropism, stomatal opening, and chloroplast relocation.

434 The leaf senescence regulator *ONSET OF LEAF DEATH 1* encodes a plant specific protein that controls multiple signaling pathways

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The onset of leaf senescence is controlled by leaf age and ethylene can promote leaf senescence within a specific age window. We exploited the interaction between leaf age and ethylene and isolated mutants with altered leaf senescence that were named as *onset of leaf death* (*old*) mutants. The *old1* mutant was selected and is represented by three recessive alleles. The senescence syndrome of *old1* was characterized using phenotypical, physiological and molecular markers and it was shown that *old1* displayed earlier senescence both in air and upon ethylene exposure. The etiolated *old1* seedlings exhibited a hypersensitive triple response. In addition, air grown mutant seedlings were hypersensitive to the plant hormones jasmonic and abscisic acid. The *old1* mutant was crossed with the *ein2, jar1* and *abi4* mutants, which have a defect in the ethylene, jasmonic acid and abscisic acid pathways, respectively, and the double mutants were analyzed. A mutation in one hormonal pathway abolished the hypersensitivity to that respective hormone, but did not affect the *old1*-specific hypersensitivity to the other hormonal pathways. This suggests that *OLD1* affect the individual hormonal pathways independent of the others. The *OLD1* gene was cloned and shown to encode a plant specific protein. The function of *OLD1* in the control of the hormonal pathways and in the regulation of leaf senescence will be discussed.

435 Cloning and Characterization of Genes Involved in Non-Photochemical Quenching in Arabidopsis

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Non-photochemical quenching (NPQ) dissipates excess absorbed light energy as heat, and thereby protects plants from high light stress. To understand the detailed mechanisms of NPQ, genes involved in NPQ were cloned by map-based cloning and reverse genetics. The *npq6* mutant was affected in NPQ, especially during the induction phase. Map-based cloning revealed that the *npq6* mutant is a frame-shift mutant caused by a single base-pair deletion in the *At5g43050* gene. Complementation of the low NPQ phenotype by *At5g43050* genomic DNA confirmed that the mutation in the *At5g43050* gene was responsible for the low NPQ phenotype of the *npq6* mutant. RACE analysis determined that the *At5g43050* gene consists of two exons and encodes a protein of 158 amino acids. The At5g43050 protein is predicted, by the TargetP program, to be targeted to chloroplasts and is also predicted to be a membrane protein with unknown function. Sequence comparisons identified two *NPQ6*-like genes in Arabidopsis: *At1g65420* and *At3g56830*. At1g65420 is annotated as a YCF20-like protein with unknown function that is conserved from cyanobacteria to higher plants including rice and barley. As a reverse genetics approach, a T-DNA insertion within the *At1g65420* gene was identified from the SALK lines, and then the interruption of gene expression was confirmed by RT-PCR. Interestingly, the knock-out of the *At1g65420* gene caused a low NPQ phenotype with very similar reduction in NPQ to that of the *npq6* mutant. To understand the function of YCF20, the *ycf20* gene in *Synechocystis* sp. strain PCC6803, sll1509, was interrupted by homologous recombination. The "sll1509 lines, in spite of a heteroplasmic state, showed reduced photosystem II (PSII) activity that was determined by 77K fluorescence and oxygen evolution. This indicates that YCF20 in *Synechocystis* is involved in PSII function or stability. To determine the function of YCF20 in higher plants, the *npq6 "At1g65420* double mutant was generated. The phenotype of the double mutant will be characterized in detail. In addition, the *npq6 "At1g65420 "At3g56830* triple mutant will be generated.

436 Physiological characterization of phytochromes with phycocyanobilin chromophores in transgenic *Arabidopsis*

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Higher plant phytochromes are composed of large apoproteins encoded by a small gene family, all of which possess phytochromobilin (PΦB) chromophores. The covalent association of PΦB is essential for the diverse photogulatory activities of phytochromes. We previously identified the *HY2* locus from *Arabidopsis* which encodes PΦB synthase (Kohchi *et al*., 2001) and the HY2-related *pcyA* gene from *Synechocystis* which encodes the biliverdin reductase, phycocyanobilin (PCB):ferredoxin oxidoreductase (Frankenberg *et al*., 2001). Through expression of plastid-targeted PcyA in the phytochrome chromophore deficient *hy2-1* mutant background, we generated transgenic plants where phytochromes possessed PCB instead of PΦB chromophores. Under white light, T1 transgenic plants (PCYA1) possessed short hypocotyls, indicating rescue of the long hypocotyl *hy2-1* mutant phenotype. Absorbance difference measurements on crude extracts from etiolated PCYA1 revealed blue-shifted spectra for the extracted phytochromes compared with those of etiolated WT seedlings. These results indicated that the phytochromes in PCYA1 plants contained PCB chromophores which could function as biologically active photoreceptors. We also analyzed PCYA1 transgenic plants for phyB-mediated red high irradiance responses (R-HIR) and low fluence responses (R-LFR), and for phyA-mediated far-red high irradiance responses (FR-HIR) and very low fluence responses (VLFR). While PCYA1 plants showed normal R-HIR, R-LFR, and VLFR activities, hypocotyl growth inhibition under high irradiances of far-red light was strongly impaired. These results indicate that the D-ring double bond of PΦB is critical for phyA-mediated far-red light signaling, as suggested by recent studies of Hanzawa *et al* (2002), implicating co-evolution of PΦB biosynthesis and phytochrome A.

Kohchi *et al*.: *Plant Cell* 13: 425-36 (2001)"
Frankenberg *et al*.: *Plant Cell*. 13:965-78 (2001)
Hanzawa *et al*.: *Proc. Natl. Acad. Sci.* USA 99: 4725-4729 (2002)

437 Molecular and genetic studies of light signaling mutants rfi2 and rfi3

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Plant photomorphogenesis is triggered by light signals, in which phyA is the sole photoreceptor to sense far-red light and phyB is the major photoreceptor to sense red light. Genetic screens have identified mutants defective either in phyA signaling or in phyB signaling. The screens have also identified two mutants, the long hypocotyl pef1 and the short hypocotyl psi2, defective in both phyA and phyB signaling. However, it is still uncertain how PEF1 and PSI2 function in both phyA and phyB signaling pathways since the two mutants are yet to be characterized at the molecular level. We have recently identified two components, RFI2 and RFI3, involved in both phyA and phyB signaling pathways. RFI2 and RFI3 are defined by their T-DNA-tagged mutants, rfi2 and rfi3 for red and far-red light insensitive 2 and 3. Rfi2 and rfi3 are not allelic to each other. Both mutants have a single T-DNA integration locus and their T-DNA insertions cosegregate with their defective light signaling phenotypes. Feeding experiments with phycocyanobilin from blue-green algae, an analog of phytochrome chromophore phytochrombilin, or biliverdin, a direct precursor for phytochrombilin, failed to rescue their long hypocotyl phenotypes. Therefore, the two mutants are not new alleles of the early-identified chromophore biosynthesis mutants, hy1 or hy2. Our future research will be directed to clone the mutated RFI2 and RFI3 genes, to conduct epistasis analysis and genetic interaction analysis of rfi2 and rfi3 with other light signaling mutants, and to study their structure-function relations and their biochemical and physical interactions with other light signaling components. In summary, the studies on rfi2 and rfi3 mutants will help us to gain valuable information on the light signaling process in Arabidopsis.

438 Carbon Supply a Pre-requisite for Plant Phosphate Starvation Response

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Phosphate (Pi) is the second most important plant nutrient that is responsible for dynamic changes in carbon fluxes. The interaction between Pi and carbon metabolism coordinates several crucial processes that control plant growth and development. In addition plant Pi levels regulate the expression of genes involved in sugar metabolism/synthesis. This close relationship between carbon and Pi initiated the current study of the effect of carbon supply on induction of phosphate-regulated genes. In this study expression of the phosphate starvation-inducible (PSI) genes in response to changes in carbon or Pi status of *Arabidopsis* was analyzed. Addition of sucrose to Pi deficient medium caused a dramatic increase in the transcript levels of the PSI genes examined. The ability of sucrose and other sugars to induce the high-affinity Pi transporter, *AtPT2* indicated that it might not be a sucrose-specific response. However, sucrose has a profound effect on gene expression. Interestingly *AtPT2* was rapidly induced within 3 h after resupply of sugars to Pi- and sucrose-starved plants. While the reporter gene luciferase under the regulation of *AtPT2* promoter, *AtPT2*-LUC was induced in Pi deprived plants supplied with sucrose, supply of the non-metabolizable sugar, 3-0-methylglucose did not lead to *AtPT2*-LUC activation. Furthermore the induction of *AtPT2*-LUC was light dependent. Transfer of light-grown plants to the dark reversed this response. Nevertheless supply of sucrose delayed the inhibition of *AtPT2*-LUC expression in the dark indicating that carbon metabolism may be required for induction of this transporter. These studies point to the potential involvement of sugars in positively regulating the expression of PSI genes in plants.

439 Cytosolic small Hsps protect plants against high temperature stress.

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Plants are vulnerable to high temperature stress at almost all stages of growth and development. To tolerate supra-optimal temperatures plants rapidly synthesize a highly conserved set of heat shock proteins. Among these, small heat shock proteins (sHsps) have been proposed to prevent irreversible aggregation of proteins during heat shock. To assess the functional importance of cytosolic Class I (CI) and Class II (CII) sHsps in plants, transgenic *Arabidopsis* plants have been developed for overproduction and inhibition (employing dsRNA) of AtHsp17.4-CI and AtHsp17.6-CII sHsps. Overexpression of these proteins did not significantly affect the normal growth and development of plants. Response of these transgenic lines to different high temperature treatments is being assessed. Growth of 2.5-d-old dark-grown hypocotyls was monitored in terms of opening of the hook and greening, after exposure to 43 degrees for 90 and 105 min. Both CI and CII sHsp-overexpressing plants exhibited enhanced hook opening and greening, whereas dsRNA lines showed a delayed response. The extent of tolerance is correlated with the level of accumulation of sHsps in all these plants. 10-d-old CII dsRNA seedlings stressed at 47 degrees and mature plants exposed to 45 degrees, after pre-conditioning, showed delayed recovery as compared to wild type and vector transformed plants. All these observations implicate Class I and II proteins in the development of thermotolerance in plants. Further, with an aim to identify proteins interacting with sHsps, the cDNA encoding AtHsp17.4-CI was fused to an affinity tag and overexpressed in *Arabidopsis* plants under the control of a constitutive or heat inducible promoter. Thermotolerance studies show that these plants possess enhanced ability to withstand heat shock as compared to wild type plants. Affinity purification of cell extracts prepared from transgenic plants subjected to pretreatment and subsequent heat stress has been carried out, followed by 2-D analysis of proteins and mass spectrometry. Several proteins including stress-related proteins, translation factors and one transcription factor have been identified as putative substrates for the AtHsp17.4-CI protein.

440 Arabidopsis ozone and light sensitive mutant *rcd2* is also defective in flowering

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Ozone is a gaseous air pollutant that is converted into reactive oxygen species (ROS) in cell walls of the affected tissues. The production of ROS is self-propagating even after the end of the initial exposure. Application of ozone to plant tissues thus creates first an O3-dependent extracellular oxidative burst, followed by subsequent plant cell-dependent, actively regulated oxidative burst. Therefore O3-exposure is a good tool to study signaling cascades that involve extracellular ROS formation in regulation of gene expression and cell death in intact plants. Here we describe an ozone and light sensitive Arabidopsis mutant, *rcd2* (for radical induced cell death2) which displays hyper-sensitivity to ozone, superoxide and avirulent pathogens, but not to hydrogen peroxide. Nearly identical concentrations of two major antioxidants, ascorbate and gluthathione in *rcd2* and Col-0 indicate that radical sensitivity of this mutant is not caused by its reduced antioxidative capacity. Also stomatal conductance, as a first defence border against ozone, is not affected in mutant. *rcd2* has significantly higher ozone induced ethylene evolution than its Col-0 wildtype. Under standard growth conditions mutant exhibits a reticulate pattern of chlorosis. Under low light conditions (<30 mmol m-2 s-1) *rcd2* is indistinguishable from Col-0 wildtype plants. In addition mutant has abberant flowering pattern since only 10-20 % of siliques develop properly and produce seed. Map based cloning of *rcd2* positioned the mutation in the bottom of chromosome two. Function of the protein mutated in *rcd2* is unknown. According to protein sequence based predictions *rcd2* has one homolog in chr. 5 and and they cluster to protein family with 8 members which all are unknown, targeted to chloroplast and have transmembrane domains.

441 Protective pathways induced during the acquisition of thermotolerance.

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The phenomenon of acquired thermotolerance, the ability of an organism to tolerate higher temperature treatments after a prior treatment at moderate temperature, is well established. Thermotolerance is not only due to the induction of heat shock proteins (HSPs): many other responses induced during moderate heating are also required for plant survival. We are using a combination of genetic and molecular techniques to investigate the signaling pathways resulting in thermotolerance. We have first assayed a number of existing mutants, including mutations in specific HSP genes, signaling components (predominantly those associated with ethylene, abscisic acid, active oxygen species and salicylic acid), fatty acid desaturases, antioxidant genes, and genes induced in other stress responses, for deficiencies in their ability to acquire thermotolerance. These mutants have been shown to be unable to tolerate heat stress at different stages in their life cycles. Very few of the mutants that show defects in thermotolerance show any reduction in HSP protein levels, therefore these genes are likely to be involved in other protective responses crucial to the development of thermotolerance at specific stages in the plant?s life cycle. In order to identify these other protective responses, full-genome microarrays are being used to determine which genes are up- or down-regulated during the acquisition of thermotolerance. The most distinct cluster of genes up-regulated in all heated samples included the HSPs and a few other protective genes. However almost 1000 genes were shown to be expressed more than 2 fold higher in thermotolerant plants than in thermosensitive plants at 45oC. These include genes for protective proteins as well as those associated with repair of intracellular machinery and breakdown of damaged cellular components, and a large number of signaling components. Levels of genes associated with photosynthesis were down-regulated in plants with acquired thermotolerance. Comparison of gene induction in mutant and wild type plants will allow specific signaling components to be associated with specific protective responses, and with protection against different kinds of heat-induced damage. This will begin to define the different pathways that together result in the thermotolerant phenotype.

442 A genetic approach to dissecting the mechanism of Hsp101 function and defining other components involved in thermotolerance

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In Arabidopsis, AtHsp101 was first described as a major component controlling acquired thermotolerance based on the phenotype of a recessive mutation in the second nucleotide binding domain (NBD2). To define further the functional domains of AtHsp101, we have analyzed 14 additional hot1 mutant alleles. Among these we identified a mutant, *hot1-4*, which is semi-dominant and not only fails to develop thermotolerance to 45^{o}C in response to pre-adaptation (38^{o}C for 1.5 hrs), but also shows impaired growth in response to the pre-adaptation treatment alone (a permissive treatment for both wt and Hsp101 null plants). The mutation (T499A) falls in the middle region between the two NBDs within a conserved motif unique to the ClpB and ClpC proteins. The semidominant phenotype of this mutant can be recapitulated by expression of the *hot1-4* mutant protein in wild type plants. We suggest that *hot1-4* affects interaction of AtHsp101 with functional partner proteins during an early step in the development of thermotolerance. *hot1-4* also exhibits a strong defect in chlorophyll accumulation and in seed germination after pre-adaptation. These observations indicate that Hsp101 functional partner proteins may be key factors for plant metabolic processes in the presence of stress. To identify additional genes involved in thermotolerance, we screened for suppressors of the *hot1-4* mutation and isolated two groups of extragenic suppressors. One group displayed a significant reversion of the *hot1-4* phenotype in pre-adaptation only, and another group showed partial reversion of the thermotolerance defect in both pre-adaptation and acquired thermotolerance. Further phenotype analyses of these suppressors could reveal factors specific to plant stress tolerance, and identify genes that encode Hsp101-interacting partners. To understand structure-function relationships within Hsp101, we also isolated nine independent intragenic suppressors of *hot1-4*. The phenotype of eight intragenic suppressor mutants after 45^{o}C heat treatment with pre-adaptation was similar to that of an Hsp101 null mutant (*hot1-3*), indicating that these intragenic suppressors eliminate the negative function of *hot1-4*, i.e. are loss-of function mutants. However, one intragenic suppressor (A297T) displayed a reversion of the thermotolerance defect of *hot1-4* in both pre-adaptation and acquired thermotolerance, suggesting either physical proximity or functional interactions between the middle region and NBD1.

443 Natural variation in the regulation of osmotically-responsive root growth

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Plant species exhibit various physiological and developmental responses when exposed to drought. One common response is to modulate root system architecture to best exploit water available to the plant. To determine the extent of natural variation in the control of root development by mild drought conditions, we scored 78 *Arabidopsis* accessions for lateral root growth under tissue culture conditions that either promote (when water is plentiful, "control") or repress (mild osmotic stress) lateral root growth in *Arabidopsis thaliana* var. Columbia (Col). We found that accessions can be grouped into three categories based on their response to these conditions. The majority of accessions behave like Col, producing branched root systems under control conditions, but making few or no lateral roots when exposed to mild osmotic stress. In contrast, a second group produces highly branced root systems under both control and mild osmotic stress conditions. A third group produces few or no lateral roots under either condition. These results indicate that *Arabidopsis* ecotypes differ in their ability to perceive and/or respond to osmotic signals. We have chosen to study Landsberg *erecta* (Ler; insensitive to mild osmotic stress) and Cape Verdi Island (Cvi; sensitive to mild osmotic stress) in greater detail because of the availability of Cvi X Ler recombinant inbred lines. We are using these RI lines to identify quantitative trait loci (QTL) that contribute to the phenotypic differences in root system architecture observed under mild osmotic stress conditions. Confirmation of the biological effects of each QTL and the map-based cloning of the gene(s) responsible should provide insight into how environmental cues are perceived and translated into changes in root morphology.

444 QTL analysis of Nitrogen use efficiency in distant accessions of *Arabidopsis thaliana*

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Improving plant Nitrogen (N) use efficiency or controlling soil N requires a better physiological and genetic characterization of the regulations of N metabolism at a whole-plant level. Our aim is to analyse the physiological regulations controlling N use efficiency and to isolate new genes involved in these regulations. This could be achieved using Arabidopsis as a model genetic system, a model that offers unequivocal genetic advantages for Quantitative Trait Locus (QTL) mapping and cloning purposes. We took advantage of the natural variation available among accessions: we used two genetically distant ecotypes, Bay-0 and Shahdara, to create a large recombinant inbred line population. Several traits (ca. 10) were measured at the vegetative stage in two contrasting N environments (contrasting nitrate availability in the soil), one of which strongly limited plant growth. We mapped a total of 117 QTL, which should represent at least 30 polymorphic genes partially controlling one or several characters. Some of these loci may correspond to known genes from the N metabolic pathway (like Glutamine synthetase structural genes), but others clearly represent new genes controlling or interacting with N physiology. For some traits, QTL x QTL epistatic interactions explain a large part of the phenotypic variation. QTL colocalizations shed new light on the relations between correlated traits (for example between total N content and shoot growth) and also between non-correlated traits, with examples of opposite relations between reduced (amino-acids) and non-reduced N compounds (nitrate). Globally, these QTL highlight the strong interaction between genetic regulations and N nutrition level. One contrasting feature has been found for shoot water content, which variation across N environments seems to share four common QTL, three of which strongly colocalize with flowering time QTL obtained in the same population. Anions contents and root architecture QTL are now mapped in this genetic material, providing a more integrated picture of whole-plant N physiology. Four loci seem particularly interesting in terms of N use efficiency variability and QTL x environment (N) interaction; the fine-mapping and candidate gene analysis of these major QTL is underway.

445 Kinase-associated protein phosphatase functions in NaCl stress signaling and adaptation

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A NaCl sensitive mutant, *rag1*(*root alternated growth*), was isolated from a T-DNA tagged Arabidopsis thaliana (C24) population. NaCl sensitivity of *rag1* is manifested primarily as a cessation of primary root growth, root tip swelling and lateral root branching. *rag1* is a loss-of-function mutation in a locus that encodes Kinase Associated Protein Phosphatase (*KAPP*, At5g19280). Growth of *rag1* seedling is indistinguishable from wild type seedlings on MS medium but exhibit root growth inhibition on medium supplemented with NaCl or LiCl but not with KCl or mannitol. *RAG1/KAPP* expression under the control of the native promoter complemented the NaCl hypersensitive phenotype *rag1*. These results indicate *rag1* is sensitive specifically to Na^+, implicating a function in ion homeostasis. *RAG1* is a negative regulator in several receptor-like kinase (RLK) signaling pathways, including those involved in brassinosteroid sensing (*BRI1*), flower development (*CLAVATA*), and innate immunity against bacterial pathogens (*FLS2*). This is the first evidence to indicate a function for *RAG1*, and perhaps a RLK signaling pathway, in salt adaptation.

446 Understanding Copper Regulation of the Metallothionein MT2a Gene Expression

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Metallothioneins (MTs) are a conserved family of small cysteine-rich proteins, found in all eukaryotes and some bacteria. Many MTs have been shown to chelate heavy metal ions and play a role in heavy metal detoxification. Transcription of many MT genes increases upon exposure to zinc or copper. The induction is mediated primarily by the metalloregulatory transcription factors ACE1 and MTF1 in *Saccharomyces cerevisiae* and in animals, respectively. *Arabidopsis thaliana* possesses seven MT homologues, some of which are inducible by copper. However, the mechanism of copper regulation in plants remains unclear. To identify the cis-acting elements that mediate copper induction of Arabidopsis MTs, fragments of the *MT2a* promoter were fused to a GUS reporter gene. GUS activity was measured in the T3 transgenic seedlings grown in MS media with or without 50 μM $CuSO_4$. Two promoter regions that are critical for copper induction were identified. The first region corresponds to the sequence from -361 to -530 nucleotides upstream of the transcriptional initiation site and is essential for the copper-induced MT2a expression at the primary root apex. A second sequence, from -131 to -192 nucleotides, is required for copper-induced expression near the root elongation zone and in the shoot. MT2a expression is also induced by oxidative stress. While copper-induced expression of the *MT2a* gene is pronounced at the root tip, induction by oxidative stress potentiators, such as paraquat or 3-aminotriazole, is observable throughout the root but with diminished intensity at the root apex. Free copper ions are known to cause production of reactive oxygen species. It is possible that copper induces the *MT2a* gene via both copper and oxidative stress signaling pathways. Experiments are in progress to locate the oxidative stress responsive elements in the *MT2a* promoter. To identify additional components that may be involved in regulation of the *MT2a* gene, an Arabidopsis line carrying the *MT2a*::GUS transgene was mutagenized with EMS. The M2 population was screened for mutants with altered level of GUS expression and/or with abnormal root growth in response to copper treatments. Analysis of the promoter deletion experiments and initial characterization of the *MT2a*::GUS mutants will be presented.

447 *HARMLESS TO OZONE LAYER* responsible for methyl halide production in *Arabidopsis*

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Methyl halides play important roles in the halogen chemistry of the atmosphere. Methyl chloride and methyl bromide are the primary carriers of natural chlorine and bromine to the stratosphere where they catalyze the destruction of ozone, whereas methyl iodide is believed to influence ozone loss in the lower atmosphere. Unlike the ozone-depleting chlorofluorocarbons (CFCs), which are produced entirely by humans, methyl halides have significant natural sources as well as anthropogenic ones. Methyl bromide is of particular interest because of its widespread use as an agricultural fumigant. However, despite the environmental and economic importance of methyl halides, their natural sources and biological production mechanisms are not well understood. Here we demonstrate, that *Arabidopsis thaliana* produces and emits methyl halides, and that the enzyme primarily responsible for the production is encoded by the *HARMLESS TO OZONE LAYER* (*HOL*) gene located on chromosome II. Plants with a disruption of the *HOL* gene have dramatically decreased methyl halide production rates compared to wild type plants. A phylogenetic analysis of the HOL sequence suggests that the ability to produce methyl halides is widespread among vascular plants. Together, the demonstration that the *HOL* gene is primarily responsible for methyl halide production in *Arabidopsis*, and the observation that such genes are widespread in diverse plant species offers a unique potential to study the importance of plants in atmospheric chemistry.

448 Expression and *in vivo* roles of V-ATPase subunit c1 and c3

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The most complex ion pump conserved in all eukaryotes is the vacuolar type H^+-ATPase (VHA). This pump is thought to acidify several endomembrane compartments of plant cells, including the vacuole, Golgi, ER, and intracellular vesicles; although the cellular and physiological consequences are poorly understood. V-ATPase consists of subunit A through H of the peripheral V_1 complex and subunit a, c, c" and d of the V_0 membrane sector. Of these, the 16 kda subunit c (VHA-c) is encoded by the largest gene family with 5 members. To analyze the cellular and physiological functions of each 16 kda isoform, we have begun to determine the expression patterns of VHA-c1 and VHA-c3, and test their *in vivo* functions by analyzing mutant plants harboring dsRNA.

Analyses of promoter::GUS expression showed that VHA-c1 is highly expressed in vascular tissues, and in hypocotyls of dark grown but not light grown seedlings. Parallel increase of VHA-c1 expression in expanding tissues points to a significant role of the V-ATPase in cell enlargement. In contrast, VHA-c3::GUS expression is restricted to a few tissues, including the root tip, anther and shoot apex. Thus, VHA-c1 and VHA-c3 genes are differentially expressed in a tissue-specific and developmentally regulated manner.

To test the *in vivo* roles of each subunit c, we have analyzed growth of plants in which VHA-c expression was reduced using double-stranded RNA-mediated interference. Dark-grown dsRNAi-c1 mutants showed a significant (38%) decrease in root length; though hypocotyl length was only 10% shorter compared to the wild type. Root length was decreased 25% in dsRNA-c3 plants. A reduction in root growth of vha-c mutants is consistent with the idea that V-ATPase activity is needed for cell division and cell expansion at the growing tip. Both *vha-c1* and *vha-c3* mutants were more sensitive than wild type plants to moderate salt stress, but not to osmotic stress. Together, these results demonstrate that V-ATPase functions *in vivo* to support root growth and confer tolerance to salt stress.

(Supported by a USDA NRI grant to HS)

Sze H, K Schumacher, ML Muller, S. Padmanaban and L Taiz. (2002) A simple nomenclature for a complex proton pump: VHA genes encode the Vacuolar H^+-ATPase. Trend Plant Sci 7, 157-161

449 *AtREV3* is involved in the error-prone DNA replication in *Arabidopsis*

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We have isolated a UV-sensitive mutant *suv1* (*s*ensitive to *UV 1*) from ion-beam irradiated *Arabidopsis* seeds, in which a gene homologous to the yeast *REV3* gene (*AtREV3*) was disrupted. The yeast REV3 is known to be involved in the error-prone translesion synthesis (TLS). That is, damaged DNA is replicated by the action of REV3 to avoid the arrest of cell division, but simultaneously REV3 allows mismatched nucleotide pair and causes replication error with high frequency. The root growth of *AtREV3*-disrupted plants was inhibited by a modest dose of UV-B in spite of the presence of normal photorepair and dark repair activities. The *suv1* plant was also sensitive to gamma-rays and MMC, which are known to inhibit DNA replication. The DNA synthesis, measured by incorporation of BrdU, in the *suv1* root cells was severely inhibited but not in the wild-type root cells by UV-B treatment. These results suggest that the DNA damage induced by UV or gamma-ray interrupts DNA replication in the *suv1* mutant, which leads to the inhibition of cell division and root elongation. We next measured the mutation frequency in the somatic cells of the wild-type and *suv1* plants. To this end, a base-substituted *uidA* gene[a] that is inactive was introduced into the wild-type and *suv1* plants. After the appropriate DNA damaging treatment, both transgenic plants were stained with X-gluc to detect blue sectors that can be seen only when the *uidA* gene reverts to active after point-mutation in the somatic cells. When the plants were irradiated with a 1kJ of UV-C, the number of blue sectors in the *suv1* plants was 20 times less than that seen in the wild-type plants. This result strongly suggests that UV-induced DNA damage is bypassed by *AtREV3* in an error-prone manner and a very large part of UV-induced base-substitution found in the *Arabidopsis* somatic cells is due to the activity of *AtREV3*.

[a]Kovalchuk, I., Kovalchuk, O. and Hohn, B. (2000) EMBO J. 19, 4431-4438.

450 Identification of mutants completing germination at low temperatures.

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Low, non-freezing soil temperature presents a barrier to the completion of seed germination thereby limiting plant production, species distribution, and direct seeding of many crops. Sub-optimal temperatures minimize degree-day accumulation prolonging the sojourn of seeds in a hostile soil environment where they are vulnerable to pathogen attack, predation, and seed-bed deterioration. We have isolated mutants capable of completing germination at 10˚C in advance of wild type from activation tagged lines of *Arabidopsis thaliana*. Following retrieval, plants from *Cold Temperature Germinating* (*CTG*) mutant- and wild type-seeds were grown under the same conditions and seeds were harvested from them on the same day for re-screening. Machine vision using automated data capture permitted wild type and mutant seeds to be assessed hourly for radicle protrusion during re-screening. These images, captured at high resolution, also permitted classification of the mutants into those also affecting seed-/seedling-size and/or seed color. Approximately half of the mutants recovered in the re-screen were BASTA sensitive. However, based on PCR results, all BASTA sensitive lines retained portions of the T-DNA including the full length ampicillin resistance gene. PCR based mapping is ongoing to determine if a functional pUC19 plasmid is inserted in these mutants. BASTA resistant lines are being crossed into wild type Columbia to obtain F2 seeds to approximate the number of insertions, assess whether the mutation of interest is tagged, and to preliminarily assess the nature of each *CTG* mutation (ectopic expression [dominant] vs insertional disruption [recessive]). Plasmid rescue, inverse- and TAIL-PCR have been used to isolate flanking DNA sequence from the mutants.

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451 DET1 & DDB1 Interact to Regulate Photomorphogenesis

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Plants are exquisitely sensitive to light, particularly during seedling development. *det1* mutants develop like light-grown seedlings even when grown in the dark. Light-grown *det1* plants exhibit additional phenotypes including excessive anthocyanin production, pale colour, dwarf stature, and lack of apical dominance. Thus DET1 is a key regulator of environmental and developmental cues. DET1 is a novel nuclear protein. An epitope-tagging strategy was used to show that DET1 functions *in vivo* as part of an ~350 kD complex. The DET1 complex was purified and MALDI-MS used to identify the primary co-purifying protein as Damaged DNA-Binding protein 1 (DDB1). DDB1 was first identified as a component of the UV-DDB complex involved in human nucleotide excision repair. The *Arabidopsis* genome contains two *ddb1* homologues, *ddb1a* and *ddb1b*. T-DNA insertional mutagenesis was used to assess the function of these genes. While loss of *ddb1b* appears to be lethal, loss of *ddb1a* results in no obvious phenotype. The DET1-interacting protein most closely matched DDB1A, so the genetic relationship between *det1* and *ddb1a* was investigated via generation of the *det1 ddb1a* double mutant. This double mutant exhibited enhanced *det1* phenotypes for all traits examined, thus *det1* and *ddb1* interact genetically as well as biochemically. *ddb1a* does not however appear to enhance the phenotypically similar *cop1-4* allele. These and other results will be presented.

452 The Identification of Novel Arabidopsis *sku* Mutants Affected in Touch Induced Cell Expansion Processes

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Plants guide the directions of root and shoot growth in response to a number of environmental stimuli including gravity and touch as well as light, water and ion gradients. In doing so, they are able to position their organs above and below ground with distributions conducive to survival. Little is known about the cell expansion processes that drive changes in directional growth. To learn more, we identified five novel Arabidopsis mutants (*sku6* through *sku10*) with roots that skewed away from the normal downward growth direction on tilted agar surfaces. These phenotypes were not the result of altered gravity responses since these mutants roots grew straight downward within an agar medium. This and other data suggest the defects lie in touch response pathways. Additionally, some of these *sku* mutants exhibited abnormal root growth responses to either one of the anti-microtubule drugs propyzamide or dithiopyr, suggesting cytoskeleton involvement. We cloned the *SKU6* gene and found it encodes a small, novel, plant specific protein. *SKU6* is allelic to *SPIRAL1* (Hashimoto, unpublished). Plants expressing a GFP::SKU6 fusion protein exhibited ubiquitous GFP-related fluorescence that likely localized to cortical microtubules. These data support a model where microtubules are involved in one or more directional cell expansion processes regulated by touch.

453 Microarray analysis of gene expression indicates that hydrogen peroxide plays a role in root sensing of potassium deficiency in Arabidopsis

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Plants must survive and grow under constantly changing environmental conditions. Changes in nutrients such as potassium (K^+) may occur as soil moisture increases or decreases, but we do not know how plant root cells sense or signal the onset of potassium deficiency. To understand how plant roots respond to K^+ deficiency at the molecular level, we conducted microarray analyses of gene expression in *Arabidopsis* roots after the plants were deprived of potassium. We used the Affymetrix GeneChip *Arabidopsis* ATH1 Genome Array. The micorarrays containing oligos of ~23,000 genes were screened with cRNA samples collected 6 h and 30 h after plants were transfered from 1.75 mM to approximately 50 µM potassium. This low concentration of K^+ created a short-term nutrient deficiency for the plants. Sixty percent of the genes on the array were detected, and the expression of over 1,000 genes was altered after potassium deprivation. The differentially expressed genes included some known potassium ion channel genes as well as transcription factors, kinases, and many metabolic genes. We found that the genes involved with reactive oxygen species production and the ethylene signaling pathway were highly induced when the plants were deprived of potassium. Moreover, H_2O_2 and ethylene production also increased after potassium deprivation *in planta*. Some of the genes whose expression increased after potassium deprivation did not increase their expression when NAPDH was inhibited. From these data, we propose that H_2O_2 may act as a signal when plants are deprived of potassium.

454 BRET: Toward a real-time live cell protein interaction assay

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Bioluminescence resonance energy transfer (BRET) is the radiationless transfer of light energy from a luciferase to a tightly associated acceptor chromophore. A physical interaction between two proteins A and B can be monitored in live cells in real time by tagging the two partners with a blue light emitting Renilla luciferase (RLUC) and the blue light absorbing yellow fluorescent protein (YFP). A shift in the luminescence spectrum of the luciferase from blue to yellow is indicative of a protein interaction. Using a RLUC-YFP fusion protein, we have established protocols for measuring BRET in stably transformed Arabidopsis as well as in tobacco BY-2 and onion epidermal cells both in vivo and in vitro. We have created cloning vectors to facilitate the expression of BRET-tagged protein fusions. BRET is now being applied to chart the protein interaction map underlying the light signaling network in Arabidopsis. BRET is a rigorous assay to confirm protein interactions in live cells and to learn how protein interactions change in response to signals in real time. We have confirmed several known as well as novel protein interactions in live plant cells. BRET promises to provide a versatile and user-friendly tool for functional genomics and proteomics in Arabidopsis.

455 Positional cloning of a dominant mutation in Arabidopsis that confers tolerance to diverse oxidative-stress conditions

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Plants encounter and adapt to a wide range of sub-optimal conditions in the natural environment. While diverse, environmental challenges such as extremes of temperature, dehydration, nutrient limitation and even atmospheric pollution, all cause a common cellular perturbation: an increase in reactive oxygen species or oxidative stress. In order to investigate processes plants use to respond and adapt to environmental stress conditions, we carried out a genetic screen to isolate mutants of *Arabidopsis* that exhibit tolerance to oxidative stress. A combination of two chemical inhibitors was found to generate the desired oxidative stress conditions on *Arabidopsis* seedlings. Aminotriazole (3-amino-1,2,4-triazole), a catalase inhibitor, and buthionine *S,R*-sulfoximine, an inhibitor of glutathione biosynthesis, were used to induce moderate oxidative stress and impair the antioxidative stress defenses. *Arabidopsis* mutant populations were screened for tolerance based on the extent of root growth under these conditions. Six mutant lines were obtained. One of the mutant lines, *oxt21*, (*ox*idative stress *t*olerant) shows striking resistance to oxidative stress and displays cross tolerance expressed as improved growth and survival under a range of different stress-inducing conditions, including growth in the presence of hydrogen peroxide, methyl viologen, acifluorfen, and enhanced tolerance to heat and drought. Genetic analysis has indicated that the *oxt21* phenotype is due to a single, dominant mutation. *Oxt21* has been mapped to an interval on chromosome I between NGA392 and GAPB molecular markers. Putative open reading frames within the identified interval was surveyed for overexpression, as no major genomic changes were detected based on a PCR analysis of the same open reading frames. One ORF shows overexpression only the mutant line under non-stress conditions. The putative function of this particular ORF may serve in a signaling role to prime *Arabidopsis* to tolerate to oxidative stress. Experimental results addressing this possibility will be presented.

456 SPA1 interacts physically with COP1 and HY5 in mediating Arabidopsis photomorphogenic development

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SPA1, a negative regulator of phytochrome A (phyA) signaling, encodes a novel, nuclear localized 114 kD protein. It contains several structural motifs: a putative Ser/Thr/Tyr protein kinase domain at its N terminus, a coiled-coil motif and two likely nuclear localization sequences in the middle and four WD-repeats at its C-terminus. The WD-40 domain of SPA1 shares high sequence homology to that of COP1, a putative E3 ubiquitin ligase. COP1 represses photomorphogenesis by direct interaction with photomorphogenesis-promoting factors such as HY5 (a bZIP transcription factor), and promotes subsequent proteasome-mediated degradation of HY5. Here we showed that SPA1 interacts with COP1 and HY5 physically in a yeast two-hybrid assay. Domain deletion studies suggested that the coiled-coil domain of SPA1 is necessary and sufficient for mediating its interaction with COP1, whereas both the WD-40 domain and the coiled-coil domains of SPA1 are required for its interaction with HY5. The overall structure of COP1 is crucial for its interaction with SPA1, and the COP1-interacting motif of HY5 is involved in interacting with SPA1. In addition, genetic studies indicated a close functional relationship between COP1 and SPA1 in repressing photomorphoigenesis. The *spa1-3* mutation strongly enhances the *cop1-6* mutant phenotype under both far-red and dark conditions. We also showed that HY5 degradation is affected in both*cop1-6* and *spa1-3* mutant seedlings under continuous far-red light. The genomic expression profiles obtained by microarray analyses focusing on the far-red light regulated genes are also consistent with these genetic observations. Taken together, our data supports the hypothesis that SPA1 acts in concert with COP1 in repressing phyA-mediated photomorphogenic responses, by helping COP1 to capture the substrates and/or modulating the proposed ubiquitin E3 ligase activity of COP1.

457 A single amino acid substitution in the Arabidopsis FIERY1 protein confers cold signaling specificity

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Most plant species in temperate regions are able to increase their tolerance to freezing temperatures by a prior exposure to low non-freezing temperatures, a process referred to as cold acclimation. During cold acclimation, many plant genes that are not expressed at normal growth temperature regimes are induced. Some of these cold-induced genes can also be activated by drought and salt stress as well as by the plant hormone abscisic acid (ABA). We previously identified a genetic locus, *HOS2*, which appears to modulate cold specific signal transduction in the activation of stress-responsive genes (Lee et al., Plant J. 17: 301). The *hos2* mutants showed an enhanced induction of stress-responsive genes by cold whereas the expression of these genes under osmotic stress or ABA treatment was not affected. In the present study, we further defined the targets of HOS2 by examining the regulation of upstream CBF transcription factor genes. It was found that the transcript levels for *CBF2* and *CBF3* were significantly higher in the *hos2* mutant than in the wild type under cold treatments, suggesting that HOS2 may act upstream of CBFs. The *HOS2* gene was cloned using a map-based strategy. Surprisingly, it turned out that the *HOS2* locus is identical to the *FIERY1* gene we had previously reported. FIERY1 is a general negative regulator that controls cold, osmotic stress and ABA signaling through regulating the turnover rates of inositol phosphate second messengers (Xiong et al., Genes Dev. 15: 1971). The *hos2* mutation rendered the HOS2/FIERY1 recombinant protein completely inactive in the cold, but did not significantly affect its activity at warm temperatures. This study provides a unique example where single amino acid substitutions could lead to conditional changes in protein functions and distinct plant phenotypes. The results may shed light on how the diversity of plant tolerance to cold and other abiotic stresses evolves due to variations in a common molecular switch.

458 Functional Analysis of BON1 Associated Proteins BAP1 and BAP2

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BON1 encodes an evolutionarily conserved calcium-dependent lipid binding protein. The *bon1* mutant is dwarf at 22?C due to reduced cell size but is wild-type looking at 28?C, indicating that it has a critical role in maintaining growth homeostasis. To further understand the mechanism of *BON1* in growth regulation, we isolated BON1 interacting proteins through a yeast two-hybrid screen. BAP1 was isolated from this screen and it contains a C2 domain and its ability to bind to phospholipids is enhanced by calcium ions. *BAP2* is a close homologue of *BAP1* with 54% sequence identity at amino acid level, and it has a weaker interaction with BON1 than BAP1 in the two-hybrid assay. Both genes are upregulated by a decrease in temperature similarly to *BON1*. In addition, they are upregulated in the *bon1* mutation, suggesting a possible feedback mechanism between *BON1* and *BAP1/2*. Genetic studies show that *BAP1* and *BAP2* have overlapping functions in cell growth control similarly to *BON1*. Overexpression of *BAP1* partially rescues the bon1 mutant phenotype. The loss-of-function mutant of *BAP1* has similar but weaker phenotype than the *bon1* mutant. It is dwarf with curly leaves at 22?C, but is wild-type looking at 28?C. The *BAP2* gene appears to have an overlapping function with *BAP1*. Overexpression of *BAP2* could rescue the *bap1* null mutant phenotype. Though the *bap2* single mutant does not have any obvious phenotype, *bap1; bap2/+* and *bap1/+; bap2* exhibit more severe growth defects than the *bap1* single mutant and the *bap1;bap2* double mutant is lethal at 22?C. Thus *BAP1* and *BAP2* may each interact with *BON1* (and possibly its homologs) to control cell growth homeostasis.

459 SGR6, a novel big protein, is involved in shoot gravitropism

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Plant shoots show negative gravitropism. We have isolated a number of *shoot gravitropism* (*sgr*) mutants of Arabidopsis to elucidate the molecular mechanism of gravitropism. The *sgr6* mutant exhibits reduced gravitropic response in the inflorescence stems and its lateral shoots grow downward. The gravitropic response is composed of four sequential steps: gravity sensing, signal formation, signal transduction, and differential growth. We have previously shown that the endodermis, which contains sedimentable amyloplasts, is essential for shoot gravitropism (Fukaki et al. 1998 Plant J. 14: 425-430). Recently, we have demonstrated that the endodermal amyloplasts failed to sediment in some sgr mutants such as *sgr2*, *sgr3* and *sgr4/zigzag* (Kato et al., 2002 Plant Cell 14: 33-46; Morita et al., 2002 Plant Cell 14: 47-56). It is inffered that these mutants are defective in gravity sensing. Interestingly, the endodermal amyloplasts sedimented in the direction of gravity in *sgr6-1* as well as in wild-type, suggesting that SGR6 is involved in the signal formation or transduction of the gravitropic response. We have cloned the *SGR6* gene based on its map position. The *SGR6* gene encodes a novel 186-kDa protein. SGR6 have no obvious conserved domain, except for two HEAT repeats, which is thought to be involved in protein-protein interaction. Investigation of SGR6 protein function during gravitropic response is in progress.

460 The regulation of the isoprene biosynthetic pathway

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Isoprene, a hydrocarbon emitted by plants is made by the isoprene biosynthetic pathway which consists of the MEP pathway and isoprene synthase. All plants have the MEP pathway but not all plants emit isoprene which is most likely due to these plants lacking the isoprene synthase gene. One hypothesis as to why plants make isoprene is thermotolerance (Sharkey and Singsaas, 1995; Singsaas et al., 1997; Sharkey et al., 2001). Studying the regulation of the isoprene biosynthetic pathway provides some insight into the evolution of isoprene synthesis and the role of isoprene in plants. The isoprene synthase gene was cloned from two isoprene-emitting species, kudzu and aspen. The isoprene synthase gene evolved from the terpene synthase family and is closely related to the monoterpene synthases of this family. The isoprene synthase protein levels were examined and correlated with isoprene emission rates in kudzu leaves that were treated with high light and/or high temperature. The transcript levels did not always correlate with the protein levels. The expression of the isoprene synthase gene suggests that light induces gene expression of isoprene synthase whereas temperature may activate the isoprene synthase protein. The expression of the MEP pathway genes in kudzu and bean, a non-emitting relative of kudzu was not different when the leaves were treated with high light and high temperature. The introduction of the isoprene synthase gene into *Arabidopsis* transformed this non-emitting species to emit isoprene in a manner that appeared to be inducible by high light and high temperature similar to kudzu. The regulation of the MEP pathway may be regulated by end-product feedback. Plants may have evolved the ability to make isoprene solely by having the isoprene synthase gene as the MEP pathway does not need to be changes.

461 Proteomics Identifies Adenosine Kinase as a Putative Player in Gravitropism

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Availability of the complete genome sequence affords Arabidopsis researchers with the ability to use a proteomics approach to identify novel candidate proteins that participate in specific pathways, such as the gravitropic response in the root tips. In this poster, a comparative proteomics approach involving the combination of two-dimensional electrophoresis (2-DE) and mass spectrometry (MS) analysis have been utilized to identify and characterize root tip proteins that are differentially represented early in response to gravi-stimulation (GS). Differential representation could derive from degradation, altered expression, cellular targeting, processing and/or modification. The root tip proteome was fractionated into sub-proteomes suitable for analysis by image software. About 1,000 protein spots combined from three sequential extractions were visible by 2-DE. Among the protein spots that were differentially represented in 3 successive experiments, we focused on 8 that increased in abundance after 12 min of GS. Interestingly, these same proteins decreased in abundance after 30 min of GS, when the elongation zone had initiated a curvature response. MALDI-TOF and/or nano-LC MS/MS were used to identify several of these differentially represented protein spots. Among them, adenosine kinase (ADK) was identified by nano-LC MS/MS and confirmed by Western blot using antibody raised against ADK. This protein was shown to participate in salvage synthesis of adenylates, methyl recycling and possibly interconverting active/inactive forms of cytokinin (Plant Physiol. 124:1775-85). Analysis of a null mutant of the *ADK1* gene revealed severe defects in gravitropic response on hard-agar surface (1.5%) and reorientation assay, but not its isomer *ADK2*. This severe phenotype may also be related to defects in root growth behavior, sensitivity to mechano-perturbation or spiral growth. Preliminary results from ADK immunolocalization analysis shows that it is highly expressed in root cap columella, quiescent center, peripheral, and tip cells but not in other regions of the root tip. Further biochemical analysis and genetic crosses with gravitropic mutants showing defects in various phases of the gravitropic response are underway to define the site of ADK function in gravity signal transduction.

462 Mutation in a Homeodomain Transcription Factor Gene, *HOS9*, Demonstrates Its Essential Role in Regulation of the Plant Growth, Development and Stress Tolerance

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To investigate the essential components mediating stress signaling in plants, we initiated a large scale screen for stress response mutants using Arabidopsis plants that carry the firefly luciferase reporter gene under the control of the stress-responsive *RD29A* promoter. Here we report the identification and characterization of one mutant, *hos9-1* (for high expression of osmotically responsive genes), in which the *RD29A::LUC* was hyperactivated by low temperature, but not by abscisic acid (ABA), or osmotic stress (NaCl). The expression of the endogenous *RD29A* gene and other stress responsive genes but not the stress-induced transcription regulator, CBF, was hyperinduced in *hos9-1* mutant plants compared to wild type plants. The *hos9-1* plants are more sensitive to freezing treatment. In addition, the *hos9-1* plants have fewer trichomes and flower later than wild type plants. The *HOS9* gene encodes a putative homeodomain transcription factor. Together, these results suggest that HOS9 mediates both plant growth, development and stress tolerance.

463 Infection by virulent *Pseudomonas syringae* alters auxin physiology in *Arabidopsis thaliana*

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Interactions between pathogenic bacteria and their plant hosts involve a complex set of signaling events that determine the success of the pathogen. One of the strategies that a pathogen may use to promote virulence and disease development is the alteration of plant physiology, for example by modulating plant hormones. We used the virulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pst*DC3000) and the host plant *Arabidopsis thaliana* as a model system to study auxin physiology during disease. In response to *Pst*DC3000 infection, Arabidopsis plants up-regulate the expression of genes encoding enzymes involved in IAA biosynthesis and the release of free IAA from IAA-amino acid conjugates. Interestingly, the induction of several of these genes during pathogen infection was dependent on the phytotoxin coronatine, suggesting that the modulation of auxin levels is a virulence strategy for *Pst*DC3000. We also observed that free IAA levels in *Pst*DC3000-infected plants are significantly higher than in uninfected plants. To explore the role of auxin during disease, we infected a variety of Arabidopsis lines that have altered free IAA levels and several auxin signaling mutants. We found that an alteration in auxin signaling affects the outcome of bacterial disease and that elevated auxin appears to be beneficial for *Pst*DC3000 virulence.

464 A Systems Biology Approach to Understanding the Arabidopsis Hypersensitive Response

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Motivation Cross-talk and feedback regulation in signal transduction networks are often quite complex. Mathematical modeling was used to help understand the *Arabidopsis* hypersensitive response (HR) to avirulent *Pseudomonas syringae*. The goal was to simulate the time course progression of the most important components of the response *in silico*. These predictions were then compared to experimental data for validation. If we truly understand the response, we should be able to get agreement with the data even for data the model has never seen.

Strategy The known measurable components of the response were taken as model variables. These included death of individual cells (PCD), salicylic acid (SA) and reactive oxygen (ROS) accumulation, and level of apoplastic superoxide dismutase (SOD). Other variables were included if predicted to be critical to system dynamics. Relationships between these variables, kinetic parameters and time delays were determined from experimental data. This process resulted in a system of ten delay differential equations governed by expert-system type rules. This system was solved numerically using engineering software (MATLAB). A one-to-one correspondence was maintained between model variables and specific signaling components. The mathematical forms for relationships between model variables also corresponded one-to-one with experimentally observed relationships between signaling components. As such, the assumptions questioned by new data can be readily identified.

Results *In silico* simulations of the time course of changes in levels of salicylic acid, PCD and hydrogen peroxide match experimental data. We have used the model to prove that direct negative autoregulation of salicylic acid biosynthesis does not exist in this system. Including terms for this extra negative feedback loop made it impossible for simulated data to match experimental results. The dynamic profiles of apoplastic superoxide dismutase (SOD) activity and two putative gene induction events have been predicted. ?Sensitivity? analysis has been used to predict which model components have the most significant influence on overall system dynamics. These predictions will aid in design of further experiments to test our knowledge of control of the HR.

465 Convergent Evolution of *avrB*-Specific *R*-Genes in Arabidopsis and Soybean

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RPM1 and *Rpg1-b* are functionally analogous disease resistance genes (*R*-genes) from *Arabidopsis thaliana* and soybean, respectively. Both confer resistance to *Pseudomonas syringae* strains that express the avirulence gene *avrB*. It is not known whether such functionally analogous *R*-genes typically result from the conservation of an ancestral specificity through speciation or whether the same specificity evolves independently in different plant lineages.

A positional approach was used to clone the soybean *Rpg1-b* gene. We have shot-gun sequenced a *Rpg1-b*-containing BAC to determine the locus structure. The BAC (114Kb) contains a total of five CC-NBS-LRR genes, *Rpg1-b* and four sequences belonging to a second family of highly related genes. Also present are two retroelements and at least four putative non-*R*-gene coding sequences. We have confirmed the identity of *Rpg1-b* both by sequencing an EMS-generated mutant allele and by functional complementation using a particle bombardment-based transient assay. In common with the Arabidopsis *RPM1* gene, *Rpg1-b* is a CC-NBS-LRR type *R*-gene. *Rpg1-b* and *RPM1* share only limited sequence similarity (29% over 600AA), most of which is confined to the NBS region. Significantly, phylogenetic analysis indicates that *RPM1* and *Rpg1-b* are not orthologous, consistent with an independent origin for these functionally analogous genes. Our observations demonstrate that *R*-genes specific for *avrB* have evolved at least twice during the evolution of land plants.

It has been shown previously in Arabidopsis that the *RPM1*-mediated response to *avrB* is dependent on an additional Arabidopsis gene, *RIN4*. Furthermore, this response can be blocked by the *P. syringae* avirulence gene *avrRpt2*. Surprisingly, *avrRpt2* also blocks the response to *avrB* in soybean plants expressing *Rpg1-b*. This observation suggests that despite their independent origins, *RPM1* and *Rpg1-b* may use related mechanisms to detect *avrB*. We are currently investigating whether a RIN4-like protein is required for *Rpg1-b* function in soybean.

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466 Testing the Guard Hypothesis: RIN4 as a common host target in resistant and susceptible pathogen interactions

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Many plant pathogenic bacteria employ the type III secretion system in order to deliver effector proteins directly into the host cell during infection. On resistant plants, some effectors can act as elicitors of defense responses by activating specific host Resistance proteins (R proteins). On susceptible hosts, these effectors contribute to virulence most likely by interacting with and manipulating host defense and signaling pathways. It has been postulated that host proteins that are virulence targets of type III effectors may also interact with R protein complexes. Thus, the effector protein could be recognized at its site of virulence. This so-called "Guard Hypothesis" predicts the existence of host proteins required during both host-resistant and host-susceptible interactions with pathogen effectors. RIN4 is a required component of the RPM1-R protein complex that recognizes the *Pseudomonas syringae* effectors AvrRpm1 and AvrB. RIN4 is also a required component of the RPS2 R-protein complex that recognizes the *P. syringae* effector AvrRpt2. Both AvrB and AvrRpm1 can physically interact with RIN4 in planta. All three effectors modify RIN4 in the absence of the respective R protein. Thus RIN4 may represent a virulence target for these unrelated type III effectors. We present genetic and biochemical evidence for a possible bi-functional role of RIN4 as a pathogen virulence target and R protein complex member.

467 Characterization of cells tolerant to *Streptomyces* phytotoxin, thaxtomin A, and isolation of thaxtomin A resistant *Arabidopsis* mutants.

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Thaxtomin A is the main phytotoxin produced by *Streptomyces scabiei*, a causal agent of the potato common scab disease. This phytotoxin is essential for *Streptomyces scabiei* pathogenicity and its exogenous application mimics the symptoms of the disease. When added to cells in suspension, this toxin induces dramatic cell hypertrophy and inhibits cell elongation. *Arabidopsis thaliana* and poplar H11-11 cells (*Populus trichocarpa* Torr.& Gray X *Populus deltoides* Marsh) were adapted to grow in lethal concentration of the toxin by progressively increasing the toxin concentration at each subculture. When compared to wild type cells, the tolerant cells present a slower growth rate, have a more spherical shape and clump into clusters. These characteristics are maintained by tolerant cells even after ten subcultures in phytotoxin free medium. Tolerant cells also show an increased resistance to two inhibitors of cellulose synthesis, dichlobenil and isoxaben. Our preliminary results suggest that thaxtomin A alters the composition or deposition of the plant cell wall. While seed germination of all plant species tested is not inhibited by thaxtomin A, developing seedling show enlarged hypocotyl, stop growing and die in a few days. We are currently screening M2 population of *Arabidopsis thaliana* EMS mutagenized plants for resistance to thaxtomin A at the level of seedling development. We have isolated so far twenty mutants with an increased thaxtomin A resistance. Phenotypic and genetic characterization of these mutants is actually in progress.

468 Expression of heat shock genes in compatible host-virus interactions

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A genetic and molecular approach is being used to understand how diverse positive-stranded RNA viruses achieve infection in *Arabidopsis thaliana*. The objective of this research is to determine the functional roles, if any, of plant-encoded heat shock genes in positive-stranded RNA virus infections. Several studies implicate heat shock proteins in various viral processes, including translation and protein folding. In addition, using microarrays we have found that the expression of a variety of heat shock genes including small heat shock proteins, *HSP70*, *HSP83*, and *HSP101* can be induced by viral infections in *Arabidopsis*. Affymetrix GeneChip analysis shows that the kinetics of expression of specific heat shock genes is differentially regulated in response to viruses. For example, we found that several heat shock genes are induced early in infection by the tobamoviruses, oilseed rape mosaic virus (ORMV) and turnip vein clearing virus (TVCV). The same heat shock genes were induced later by cucumber mosaic virus (CMV), potato virus X (PVX), and turnip mosaic potyvirus (TuMV). Furthermore, the microarray data demonstrated that all heat shock genes are not coordinately regulated in response to infection by a given virus. We have made these observations at both the mRNA and protein levels. In particular, *HSP101* mRNA and protein expression peaked at 2 days after inoculation and declined to low detectable levels by 3 days after ORMV inoculation. In contrast *HSP17.6* mRNA declines, but is still detectable, while its protein levels increase over a 4-day time course. These results indicate that heat shock gene expression is not the result of a general stress response but that it may be controlled by virus-specific mechanisms. The expression of heat shock genes during virus infection suggests that they could have a role in viral pathogenesis. To test this possibility, mutants for *HSP101* and *HSP17.6* are being used to determine functional roles, if any, of these proteins in ORMV infection. ORMV accumulation in these mutants will be presented.

469 Comparison of type III effector collectives identified from multiple *Pseudomonas syringae* pathovars via a near-saturating, high-throughput screen

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We are interested in identifying the host targets manipulated by pathogens during successful infections. We work on the model interaction between Arabidopsis and pathovars of *Pseudomonas syringae*. As a first step towards our goal, we are identifying and comparing the suite of type III effectors from multiple strains of *Pseudomonas syringae* pathovars. Fourteen pathovars have been selected based on the phylogenetic analysis of Sawada et al (1999), their diversity in host range, and their evolutionary distance. To facilitate this immense project, we created a four-step, high throughput, near saturating screen. It is based on 1) automated cloning of HrpL-induced genes using a fluorescence activated cell sorter (FACS), 2) identification of hrp-boxes using a Hidden Markov Model, 3) infiltration of pools of clones, derived from their respective contigs to identify which encode proteins capable of delivering the C-terminal portion of AvrRpt2 into Rps2-expressing Arabidopsis plants and, 4) isolation of the complete ORF/operon corresponding to the HrpL binding site. Several genomes can be screened in parallel, yielding their effector genes in less than three months, with minimal labor. We present our screen and analyses of genes identified from the genomes of three pathovars. Our collection of effector genes and their alleles will provide the biological tools for identifying their respective host targets, how these targets are manipulated for the benefit of the pathogen and, insight into how a host becomes amenable to pathogenesis.

470 Characterization of the avrBs1 locus of X. c. pv. campestris

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Xanthomonas campestris pv. *campestris*(Xcc) causes black rot diseases on crucifer plants. Like many other Gram negative bacterial pathogens, Xcc injects an array of virulence proteins, referred to as type III effectors, into the host cells through the type III secretion system. Here we report the characterization of the *avrBs1* locus of the Xcc. The Xcc *avrBs1* locus contains two genes, *ORF2099* and *ORF2100*, that appear to encode two type III effectors. *ORF2099* encodes a functional phosphotyrosine phosphatase that is homologous to HopPtoD2, a type III effector of *Pseudomonas syringae* pv. *tomato* DC3000 strain. *ORF2100* encodes a protein homologous to the C-terminus of the AvrA protein of *P. s*. pv. *glycinia* and accounts for the avirulence activity in *X. c.* pv. *vesictoria* on pepper plants carrying the *Bs1* disease resistance gene. Insertion mutation of *ORF2099*, which also created a polar mutation on *ORF2100*, in Xcc 33913 strain clearly reduced the bacterial pathogenicity on cabbage and Arabidopsis plants. Disruption of *ORF2100* alone in Xcc also reduced the bacterial pathogenicty. Expression of ORF2099 alone in Xcc slightly enhanced the bacterial pathogenicity. These results suggest that both ORFs contribute to bacterial pathogenicity. *ORF2099* alone does not have avirulence activity on the Bs1 pepper plants. However, a nonsense mutation that truncates the *ORF2099* genes reduced the avirulence activity of the *avrBs1* locus, suggesting a functional interaction of the ORF2099/2100 proteins in avirulence function. Point mutation that specifically knocks out the phosphotyrosine phosphatase catalytic site of ORF2099 reduced the avirulence activity more than did by the ORF2099 nonsense mutant, suggesting the involvement of the tyrosine phosphatase activity and the dominant negative role of the mutant protein in avirulence function. Further studies focuses on the interaction of the of ORF2099/2100 proteins, the role of the tyrosine phosphatase activity of ORF2099 in virulence and avirulence, and the molecular functions of ORF2099/2100 in plant cells.

471 Mutants with reduced responsiveness to effector AvrB

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RAP2.6 is a member of ERF (Ethylene Responsive Factor) transcription factor super family. We report that the transcription of *RAP2.6* is closely associated with disease susceptibility and bacterial virulence. To understand the molecular mechanisms underlying bacterial diseases, we generated a *RAP2.6-LUC* transgenic line to monitor plant responses to various *Pseudomonas syringae* virulence factors including several effector genes and phytotoxin. *RAP2.6-LUC* transgenic seeds were mutagenized with EMS, and 15,000 M2 plants were screened for mutants that showed reduced *RAP2.6* promoter activity in response to the effector gene *avrB* carried by *P. syringae*. At least seven mutants were isolated. These mutants are tentatively called rrb mutants for reduced responsiveness to *avrB*. Here we report the characterization of two such mutants, *rrb17*,and *rrb284*. Genetic analyses indicated that they were caused by recessive mutations at two distinct loci. Both *rrb17* and *rrb284* displayed ethylene insensitivity and enhanced disease susceptibility to *P. s.* tomato DC3000, supporting a role of ethylene signaling in plant defense or bacterial virulence. *rrb284*,but not *rrb17*,also showed enhanced susceptibility to *Botrytis cinera*. Cloning and further characterization of these mutants are in progress.

472 Identification of Potential Components of the EDR1 Kinase Pathway

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EDR1 was identified in a screen for Arabidopsis mutants with enhanced resistance to pathogens, including the fungus *Erysiphe cichoracearum* (Frye and Innes, 1998). The enhanced resistance observed in *edr1* is characterized by failure of the fungus to produce conidiophores and the formation of necrotic regions surrounding the site of infection in the leaf. These lesions are formed approximately seven days after infection and may restrict the growth of the fungus by limiting nutrients. In addition to enhanced resistance, the *edr1* mutant also appears to be altered in its response to certain plant hormones. When treated with ethylene, adult *edr1* plants are more sensitive than wild type and begins to senesce earlier (Frye et al., 2001). When treated with cytokinin, *edr1* plants are less sensitive and continue to senesce while wild type does not. These responses suggest that *EDR1* may be a negative regulator of cell death in response to pathogen infection and hormone treatment. *EDR1* was cloned and encodes a kinase that is similar to CTR1 and four other kinases from Arabidopsis. To identify potential targets of EDR1 phosphorylation, a yeast two-hybrid library screen was performed. To increase the stability of potential EDR1-substrate interactions, the catalytic site of EDR1 was mutated to block the phosphotransfer reaction, which is thought to reduce the dissociation rate of substrates.. Two promising interactors have been identified and will be discussed in more detail on the poster. This research is supported by NIH grant R01 GM63761.

473 Direct physical interaction between RRS1-R, an Arabidopsis thaliana TIR-NBS-LRR-WRKY protein conferring broad spectrum resistance to Ralstonia solanacearum and PopP2, a type III effector

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iRRS1-R confers broad spectrum resistance to several strains of the causal agent of bacterial wilt, *Ralstonia solanacearum*. Although genetically defined as recessive, this novel R gene that encodes a protein whose structure combines the TIR-NBS-LRR domains found in several R proteins and a WRKY motif characteristic of some plant transcriptional factors, behaves as a dominant gene in transgenic susceptible plants. We identified *PopP2*, a *R. solanacearum* type III effector which belongs to the YopJ/AvrRxv protein family, that corresponds to the avirulence protein perceived by *RRS1-R*. A physical interaction between PopP2 and RRS1-R has been demonstrated using the yeast split-ubiquitin two-hybrid system. This interaction that requires the full-length R protein was not observed between the RRS1-R protein and PopP1, another *R. solanacearum* member of the YopJ/AvrRxv family. Both the PopP2 and the RRS1-R proteins colocalize in the nucleus. Data showing that the nuclear localization of the RRS1-R protein is dependent on the nuclear targeting of PopP2 will be presented.

474 Expression profiling of defense responses during compatible interactions

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Although many signal transduction intermediaries that control plant responses to pathogens have been identified, gaps remain in our understanding of this complex network and the interactions among its constituent pathways. To identify the complement of genes that are transcriptionally regulated in response to pathogen infection, we are doing full-genome expression profiling of Arabidopsis using the Affymetrix ATH1 GeneChip, which consists of probe sets representing approximately 24,000 genes. Because pathogens with different modes of infection are known to induce unique sets of responses, we are assaying the transcriptional effects of infection by different classes of fungal pathogens, including the biotrophic pathogen Golovinomyces orontii (also named Erysiphe orontii), which grows on epidermal surfaces and is a causal agent of powdery mildew; the necrotrophic pathogen Botrytis cinerea, which causes soft rot; and Fusarium oxysporum, a vascular pathogen which causes wilt. Examination of data obtained so far with G. orontii and B. cinerea has lead to identification not only of genes that are induced or repressed by both pathogens but also of a group of genes that are induced in response to B. cinerea but repressed by G. orontii infection. We have developed a database (Plant-Microbe Interaction Database, or PMIDB) for storage of microarray data which will be publicly accessible via Web interface. Incorporated into the design is the capability to store raw and normalized data from both Affymetrix GeneChips and spotted arrays as well as MIAME-compliant experimental information. When fully operational, data can be retrieved using various search criteria and exported. Our goal is that PMIDB will be a community repository of expression profiling data for plant-pathogen interactions, and toward this end we invite other groups to contribute data. This project is funded by an NSF Arabidopsis 2010 Grant (project #0114783) to FMA (co-PI) and collaborators Dr. Xinnian Dong (PI) and Dr. Shauna Somerville (co-PI).

475 Fusarium wilt disease of Arabidopsis

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A wilt disease of *Arabidopsis thaliana* can be instigated by pathogenic forms of the fungus *Fusarium oxysporum*. We have begun to characterize the genetic interaction between Arabidopsis and Fusarium by examining the resistance and virulence, respectively, of both plant and fungal mutants. Arabidopsis ecotypes display varying degrees of resistance to Fusarium. We have identified at least three dominant traits that contribute to resistance. A number of eds mutants, which were isolated for enhanced susceptibility to the foliar pathogen *Pseudomonas syringae*, are also more susceptible to Fusarium root disease. This suggests that the defects in such eds mutants have defects in general regulators of disease resistance. Easy and routine genetic transformation of the fungal pathogen and the available molecular tools for Arabidopsis makes the genetic analysis of both the host and pathogen possible.

476 Identification of *srf* (suppressor of *rps4-RLD*) mutants that exhibit *avrRps4*-specific disease resistance

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RPS4 is a TIR-NBS-LRR disease resistance gene in Arabidopsis that specifies the disease resistance response to *Pseudomonas syringae pv. tomato* expressing *avrRps4*. *RPS4* was cloned based on the identification of RLD as a naturally occurring susceptible accession. Comparison of the non-functional *rps4-RLD* allele with functional alleles of Col-0, Ler, Ws-0 and Po-1 showed no drastic changes in the open reading frame, even though RLD displays full susceptibility when comparing inoculations with virulent and avirulent bacteria. Subsequently, a single amino acid change (N195D) at a non-conserved position in the *rps4-RLD* NBS domain was shown to abolish RPS4 function. To dissect the molecular and genetic basis of disease resistance, we are using a genetic approach to identify suppressor mutations that reactivate the *RPS4*-triggered plant defense response in RLD. We have completed the screening of 114 M2 pools of EMS-mutagenized RLD and have identified several *srf* (suppressor of *rps4-RLD*) mutants that display *avrRps4*-specific resistance. After back-crossing to the parental RLD line, we have to date verified two recessive *srf* mutants that fall into separate complementation groups. Ongoing experiments designed to further characterize these mutants include: (i) RNA gel blot analysis to examine expression levels of defense genes in *srf* mutants, (ii) bacterial growth analysis within leaf tissue of *srf* mutants, (iii) genetic analysis and map-based cloning of suppressor genes, (iv) epistasis analyses between known disease resistance (*eds1-1* and *NahG*) and *srf* mutants.

477 Analysis of the role of RPS2-interacting proteins in disease resistance

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The *Arabidopsis thaliana RPS2* gene confers resistance to strains of *Pseudomonas syringae* that express the AvrRpt2 effector protein. RPS2 belongs to the CC-NBS-LRR class of R gene products that carry a coiled coil (leucine zippper), a nucleotide binding site, and leucine-rich repeats. To elucidate the process by which RPS2 detects AvrRpt2 and initiates defense, we used a modified yeast two-hybrid system to identify twelve Arabidopsis proteins that interact with RPS2. These interactor proteins may represent virulence targets of AvrRpt2, and/or proteins that participate in plant defense signal transduction. To determine whether these proteins are required for RPS2-mediated gene-for-gene resistance, we are identifying homozygous T-DNA insertion lines for each gene. We will discuss the potential role of these proteins in RPS2-mediated resistance, based on the resistance phenotype of their T-DNA insertion lines and related experiments.

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478 Activation of RPS5-mediated resistance via cleavage of PBS1 by AvrPphB

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Induction of plant disease resistance has long been thought to occur after direct recognition of a specific pathogen factor by a matching plant resistance (R) protein. Little evidence supports this hypothesis. Our results support an alternative model, the Guard Hypothesis, where R proteins, which guard pathogen virulence targets, are indirectly activated upon the modification of these virulence targets by their corresponding pathogen factors. We have previously shown that the *Pseudomonas syringae* type III effector AvrPphB is a cysteine protease that must process a plant substrate to induce disease resistance in Arabidopsis plants carrying the *R* gene *RPS5*. Here, we identified a direct plant substrate of AvrPphB. This substrate, PBS1, is required for *AvrPphB/RPS5*-specified resistance, suggesting that a proteolytic product of PBS1 is the signal detected by RPS5 to activate resistance.

479 Characterization of the Suppression of HR by Turnip Crinkle Virus

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TCV, in common with many animal viruses, is able to suppress the defensive programmed cell death response of the host. This response in plants is called the hypersensitive response (HR). We have shown that TCV is capable of suppressing the HR to both a viral (TCV coat protein) and a bacterial (avrRpt2) avr in Arabidopsis. Prior to HR formation, there is typically a strong oxidative burst, producing among other reactive oxygen species, hydrogen peroxide. This oxidative burst is thought to be a signal for the HR. A fluorescent detection method was used to measure the hydrogen peroxide produced in HR-suppressed Arabidopsis when challenge-inoculated with *Pseudomonas syringae* pv. *glycineae* R4 carrying avrRpt2 or an empty vector. In non-HR suppressed plants, the avr-expressing bacteria resulted in a 2-3 fold differential in hydrogen peroxide production. This differential was almost completely eliminated in HR-suppressed plants. A similar reduction in the differential induction of *PR-1* was seen in HR-suppressed plants. The presence of TCV, however, did not change the growth of virulent or avirulent bacteria in infiltrated plants. Experiments to determine the causative agent of the suppression have ruled out the viral CP and movement proteins as being individually sufficient.

480 A novel signaling component involved in the regulation of NHO1

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We recently showed that *NHO1* is an innate immunity gene required for plant resistance to nonhost *Pseudomonas* bacteria. Interestingly, the expression of *NHO1* is induced by nonhost *Pseudomonas* bacteria but suppressed by a virulent bacterium *P. syringae* pv. *tomato* DC3000. These suggest that the signaling pathway controlling *NHO1* expression is actively modulated by both organisms for defense or parasitism. In order to gain insight into signaling mechanisms underlying plant innate immunity, we characterized a novel mutant designated *rrb129* that was identified from an independent mutant screen (see abstract of Chintamanani et al.). The *rrb129* mutant was caused by a single recessive mutation nonallelic to *NHO1* and displayed reduced *NHO1* expression in response to *Pseudomonas* bacterial infection. Significantly, the *rrb129* plants were highly susceptible to nonhost bacteria *P. syrinagae* pv. *tabaci*, consistent with a role in nonhost resistance. *rrb129* is also completely susceptible to avirulent bacteria and displayed enhanced disease susceptibility, suggesting a crucial role of the *RRB129* gene in disease resistance. Further characterization of this mutant will be described.

481 Control of defense gene expression in compatible host-virus interactions

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We previously established that several positive-stranded RNA viruses elicit the expression of common sets of genes in susceptible Arabidopsis plants. Approximately one-third of the genes induced in common by these viruses can be associated with plant defense and stress responses. We wanted to know if the expression of defense-related genes in compatible host-virus interactions is regulated through signaling pathways that normally control plant defense responses. To answer this question, *pad4-1*, *eds5-1*, *NahG*, *npr1-1*, *jar1-1*, *ein2-1*, and wild type Columbia-0 plants were infected with two different viruses, cucumber mosaic cucumovirus (CMV) and oilseed rape mosaic tobamovirus (ORMV). RNA was extracted from inoculated leaves at 2 and 5 days after inoculation. Northern blots were probed with labeled DNA fragments corresponding to *beta-1,3 glucanase 2* (*BGL2*), *PR5-like* (At1g75040), and *PDF1.2 genes*. *PDF1.2* was not expressed in response to any virus treatment as expected based on previous microarray data. The *BGL2* and *PR5-like* genes were induced by CMV and ORMV at 5 days after inoculation in Columbia-0, *ein2-1*, *eds5-1*, *jar1-1*, and *npr1-1*. Expression of *BGL2* and *PR5-like* genes was reduced in *pad4-1* and abolished in *NahG* transgenic plants. Our preliminary results indicate that expression of defense-related genes in susceptible plants is salicylic acid-dependent, but *npr1-1-*, *jar1-1-*, and *ein2-1*-independent.

482 Identification of a putative plant receptor for the *Agrobacterium* T-pilus

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Agrobacterium tumefaciens transforms plant cells by transferring T-DNA from the bacterium to plants and integrating it into the nuclear genome. The transfer mechanism shares many features with conjugal DNA transfer between bacteria. The T-DNA export apparatus is composed of two major structural components: the T-pilus and a membrane associated complex that is responsible for translocating substrates across both bacterial cell membranes. The major component of the T-pilus is VirB2 protein. In *Agrobacterium*, this protein is processed and cyclized before assembly. Although the T-pilus is essential for T-DNA transfer and virulence, understanding its specific mechanistic functions remains a challenge. Using the C-terminal, processed portion of VirB2 protein as a bait in a yeast two hybrid screen of an *Arabidopsis* cDNA library, we identified three related plant proteins, VirB2 Interacting Protein (BTI) 1, (BTI1), BTI2 and BTI3, with unknown functions, and a membrane-associated GTPase, AtRAB8. The three BTI proteins also interact with VirB2-GST fusion proteins in GST pull-down assays. To understand the possible function of these plant proteins, we generated both anti-sense and RNA interference transgenic plants of BTI1, BTI2 and AtRAB8. Based on stable and transient transformation assays, both anti-sense and RNA interference transgenic *Arabidopsis* plants of BTI1, BTI2 and AtRAB8 were less susceptible to *Agrobacterium* transformation than were wild-type plants. *Arabidopsis* mutant plants containing a T-DNA insertion either in the upstream region or the downstream region of the BTI1 gene were recalcitrant to *Agrobacterium* transformation. BTI1 RNAi plants also showed altered root morphology. Root hairs were “bulbous” or “branched”. Finally, GFP fusions with BTI1, BTI3, and AtRAB8 were generated in transgenic plants to study their cellular localizations. Preliminary confocal microscopic data indicate that the AtRAB8 (GTPase) localizes throughout the cytoplasm, whereas BTI1 and BTI3 preferentially localize to the cell periphery.

483 Jasmonic acid signaling mutants of Arabidopsis exhibit reduced susceptibility to *Pseudomonas syringae*

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We are interested in disease susceptibility and the mechanisms by which virulent pathogens modify normal host cell processes to promote tissue colonization and disease development. In a screen for Arabidopsis mutants with reduced susceptibility to the bacterial plant pathogen *Pseudomonas syringae* pv. *tomato (Pst)* we isolated an allele of the coronatine insensitive locus (*COI1*). *coi1* mutants are insensitive to the plant hormone methyl jasmonate (JA) and to the bacterial phytotoxin coronatine, a *P. syringae* virulence factor believed to act as a molecular mimic of JA. We have demonstrated that reduced susceptibility to *Pst* in *coi1* plants is correlated with sensitization of the salicylic acid (SA)-dependent defense response pathway and that that SA-mediated defenses are required for restriction of *Pst* growth in *coi1* plants. To determine whether reduced susceptibility is a common feature of JA signaling mutants, we have assayed disease susceptibility in several other JA signaling mutants, including *jin1*, *jar1* and *axr1* . *jin1* and *axr1* mutants exhibit reduced disease susceptibility to *Pst*, whereas *jar1* mutants exhibit normal levels of disease. To learn more about the role of JA signaling in *P. syringae* pathogenesis we are investigating the genetic and molecular basis of reduced susceptibility in *jin1* plants. Similar to the *coi1* mutant, reduced susceptibility to *P. syringae* in *jin1* is correlated with insensitivity to coronatine and elevated *PR* gene expression, and is dependent on SA. Thus, an intact JA signaling pathway is required for full susceptibility to infection by virulent *P. syringae*, and may serve as a virulence target through which the pathogen can modulate plant defense.

484 Chlorophyllase - a central enzyme in Chl-degradation and pathogen defense

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The gene for chlorophyllase is induced in responses to the bacterial necrotroph *Erwinia carotovora*. To elucidate the role of chlorophyllase in the defense responses of Arabidopsis we generated transgenic plants with RNAi silenced and overexpression constructs of the *AtCLH1*gene. Silencing of the gene led to enhanced resistance to *E. carotovora* when the plants were infected in normal light conditions but not at low light. The inability to degrade chlorophyll resulted in oxidative stress manifested as strong accumulation of hydrogen peroxide (H2O2) in the *E. carotovora* infected plants. This was accompanied by induction of SAR marker genes *PR1* and *PR2*, as well as the antioxidant defense marker gene *GSTI*. The damage caused during a pathogen attack in normal light conditions in combination with the inability to degrade chlorophyll efficiently creates oxidative stress that in turn triggers the SAR-pathway creating resistance to *E. carotovora*.

485 Induction of Arabidopsis glucosinolates by Myzus persicae (green peach aphid) feeding

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Glucosinolates (GS) and their breakdown products are defensive secondary metabolites in Arabidopsis and other Crucifers. Generalist insect herbivores such as Myzus persicae (green peach aphid) tend to be sensitive to GS. Feeding by M. persicae significantly induced GS in Arabidopsis ecotype Columbia leaves and reduced plant weight compared to plants without aphid treatment. Ten aphids were placed on a leaf in a clip cage for 3 days, and GS levels in local (with aphids) and systemic (no aphids, no cage) leaves were measured by HPLC-MS. Control plants received cages without aphids. Although GS levels varied with the developmental stage of the plant, major GS found in Columbia leaves were significantly more abundant in both local and systemic leaves than in the control leaves. On a flowering plant, more than 80% of aphids were found on the flower stalk and/or on newly developing leaves. When Columbia plants had a 5-10 cm flower stalk, two phenylalanine-derived GS, indolyl-3-methyl-GS and 4-methoxy-indolyl-3-methyl-GS were higher in rosette leaves than in the flower stalk and newly developing leaves, whereas two aliphatic GS, 4-methylsulfinylbutyl-GS and 8-methylsulfinyloctyl-GS, were lower in leaves. In particular, 4-methoxy-indolyl-3-methyl-GS in various parts of the inflorescence was less than 10% of the leaf levels. Further work will determine the effects of particular induced GS varieties on aphid feeding preferences and fecundity.

486 The opposing effects of light signals on leaf blade and petiole growth

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Leaf photomorphogenesis is a complex developmental event. As the major photosynthetic organ, leaves are required to optimize their shape in response to light conditions. Shade not only induces elongation of the petiole, but also reduces expansion of the leaf blade. In other words, the shade avoidance responses of the leaf blade and petiole differ, suggesting that differential control of leaf blade and petiole development is key to leaf photomorphogenesis. However, little is known about the genetic mechanisms that control environmental plasticity in leaf photomorphogenesis. This study evaluated the effects of light on leaf blade and petiole growth using photoreceptor mutants. Under various monochromatic light conditions, the *phyA*, *phyB*, and *cry1* mutants and *phyAphyB* double mutant showed reduced shade-avoidance syndrome compared with the wild type. This indicates that these photoreceptor signals are involved in controlling the differential growth of the leaf blade and petiole. Interestingly, the promotor effect of blue light on leaf-blade elongation required a high fluence rate. To examine the role of photoassimilates in promoting leaf-blade expansion, photosynthesis was inhibited by applying 3-(3',4'-dichlorophenyl)-1,1'-dimethylurea (DCMU) and exposing the plants to various kinds of light. Consequently, the stimulatory effect of blue light on elongation was reduced. A supply of sucrose reversed the effect of DCMU, suggesting that sucrose produced by photosynthesis is essential for the effect of blue light on elongation of both the leaf blade and petiole. The effects of these factors on the differential growth of leaf blades and leaf petioles are discussed. We also examined the role of leaf morphogenesis genes, such as *ROT3*, and phytohormones, such as auxin and ABA, on the photomorphogenesis of leaves. Our results indicate that phytohormones such as brassinosteroids, auxin, and ABA have a role in organ-specific regulation of the leaf blade or leaf petiole. The interaction of phytohormones with signals from various photoreceptors is discussed in terms of the differential regulation of the leaf blade and petiole.

487 WRKY70: a Node of Convergence for JA/SA-mediated Signals in Plant Defense

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Cross-talk between salicylic acid- (SA) and jasmonic acid- (JA) dependent defense signaling has been well documented in plants, but how this cross-talk is executed and what are the components involved remain to be elucidated. We demonstrate that the plant specific transcription factor WRKY70 is a common component in SA- and JA-mediated signal pathways. Expression of *WRKY70* is activated by SA and repressed by JA. The early induction of *WRKY70*by SA is NPR1-independent but functional NPR1 is required for full-scale induction. Modulation of *WRKY70* transcript levels by constitutive overexpression increases resistance to virulent pathogens and results in constitutive expression of SA-induced pathogenesis-related genes. Conversely, antisense suppression of *WRKY70* activates JA-responsive/COI1-dependent genes. The effect of WRKY70 is not caused by subsequent changes in SA or JA levels. We suggest that WRKY70 acts as an activator of SA-induced genes and repressor of JA-responsive genes integrating signals from these mutually antagonistic pathways.

488 The role of *CPNI* in disease resistance in Arabidopsis

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Previously, we identified an Arabidopsis copine mutant, *cpn1-1*, that has a humidity and temperature dependent lesion mimic phenotype with increased resistance to *Pseudomonas syringae pv. tomato (P. s. t.)* bacteria, an accelerated HR and constitutive PR gene expression. This phenotype suggests that *CPN1* may act as a suppressor of defense-related cell death and other defense responses. In this study, we carried out a gene expression analysis of *CPN1*. In wild type plants, *CPN1* transcript accumulation was rapidly, locally and transiently induced by both avirulent and virulent strains of *P. s. t.* However, the induction of *CPN1* transcript accumulation by avirulent bacteria was much faster and stronger than that induced by virulent bacteria. In addition, double mutant analyses showed that both *cpn1-1/npr1-1* and *cpn1-1/eds5-1* double mutants had an intermediate phenotype as compared to the parent lines, suggesting that the *cpn1-1* mutant phenotype may be partially independent of *NPR1* and *EDS5*. Taken together, these results suggest that *CPN1* is pathogen regulated and could function partially independently of *EDS5* and *NPR1*.

489 *Arabidopsis thaliana* and *Brassica ssp.* responses to the different cauliflower mosaic virus genes.

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Cauliflower mosaic virus (CaMV) infection produces a wide range of responses in cruciferous plant species. Various *Brassica* species respond differently to CaMV infection. *Brassic rapa* (AA) and *Brassica nigra* (BB) are susceptible to CaMV infection, showing severe symptoms with high accumulation of the viral DNA. *Brassica oleracea* (CC) responds initially with mild transitory symptoms and then plants recover from infection. This recovery has been associated with post-transcriptional gene silencing within the virus genome. In addition it has been observed in *Brassica oleracea* and *Brassica napus* (AACC) that introduced transgenes with sequence homology to CaMV also show gene silencing (Covey *et al.* 1997, Al-Kaff *et al.* 1998 and 2000).

Arabidopsis response to CaMV infection is similar to *Brassica rapa*, by developing a wide range of systemic symptoms. We have previously screened 166 ecotypes of *Arabidopsis* and found that they were susceptible to CaMV infection, but symptom types and severity differed from one ecotype to another. It was found that *Arabidopsis* transformed with only Gene VI of CaMV exhibits a range of symptom-like-phenotypes (Cecchini *et al.* 1997). To study individual CaMV gene functions in *Arabidopsis*, constructs have been made with different CaMV genes (I, II, III, IV, V and VI), these constructs will be transformed into different ecotypes of *Arabidopsis*.

Cecchini, E., Gong, Z., Geri C., Covey, S.N. and Miller, J.J. 1997. Mol. Plant-Microbe Interact 10: 1094-1101.
Covey, S.N., Al-Kaff. N.S., Langara, A. and Turner, D.S. 1997. Nature 835: 781.
Al-Kaff, N.S., Covey, S.N., Kreike, M., Page, A., Pinder, R. and Dale, P. 1998. Science 279: 2113-2115.
Al-Kaff, N.S., Kreike, M., Covey, S.N., Pitcher, R., Page, A.M., Dale, P.J. 2000. Nature Biotechnology 18: 995-999

490 Genetic control of Arabidopsis defense responses to Botrytis cinerea

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Botrytis cinerea is a necrotrophic fungal pathogen that causes pre-and post harvest diseases in many crops under diverse production conditions. The molecular and genetic basis of host response to Botrytis is poorly understood. A direct genetic screen was performed to identify the genetic components of plant defense responses to Botrytis. Four Arabidopsis mutants with enhanced susceptibility to Botrytis (bos) were identified. Interestingly, three of these mutants also show increased susceptibility to another necrotrophic pathogen, Alternaria brassicicola indicating common mechanism of resistance. The BOS1 gene was cloned based on a T-DNA insertion in the gene and encodes an R2R3MYB transcription factor protein. The other three loci defined by the bos2, bos3, and bos4 mutants are not tagged by the T-DNA insertion. The bos mutants exhibit both overlapping and distinct aspects of their phenotypes. The bos1 and bos2 plants show wild type levels of susceptibility to the virulent strains of the oomycete pathogen Pernospora parasitica and the bacterial pathogen Pseudomonas syringae pv. tomato. In contrast, bos3 plants show resistance response to these pathogens resulting in reduced pathogen growth in infected tissues. Interestingly, bos3 plants are hypersensitive to cold stress. Exposure of bos3 plants to 40C temperature for 48 hours caused extensive and distinct chlorosis and yellowing that progressively covers the entire leaf area. The Botrytis susceptibility phenotype is the only altered response of bos2 observed in the various assays performed so far. The bos mutants provide novel avenues for the molecular dissection of host response to Botrytis and other necrotrophic pathogenes. Data on the molecular and genetic analysis of the BOS loci, and their relationship to other diseases and stress response pathways will be presented.

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491 Unraveling avirulence from virulence: A possible role for *Pseudomonas* avirulence effectors as suppressors of plant defense responses.

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Plant pathogenic bacteria harbor a number of so called avirulence (*avr*) genes, which have been identified by their ability to induce a hypersensitive response (HR) in plants that express the corresponding resistance (*R*) gene. Although *avr* genes are assumed to contribute to virulence on susceptible host plants, a role in virulence has only been documented for a handful of *avr* genes and the biochemical function of most Avr products remains undefined. Using the pathogen *Pseudomonas syringae* pv *tomato* DC3000 (DC3000), we have been working to determine the virulence function of AvrPphB and two DC3000 homologs AvrPpic2 and Orf7. We are also working with a third AvrPphB homolog, Orf4, derived from *P. syringae* pv *phaseolicola*.

Recently we have shown that AvrPphB functions as a cysteine protease, and that protease activity is required for induction of resistance responses in plants containing a matching *R* gene (1). On Arabidopsis genotypes lacking a corresponding R gene, however, AvrPphB appears to suppress defense responses. Specifically, Northern blot analysis has shown that AvrPphB delivered by DC3000 suppresses induction of PR1 expression in susceptible Arabidopsis genotypes. We are currently assessing whether this suppression requires AvrPphB protease activity.

We have generated *avrPpiC2* and *orf7* single and double knockouts in DC3000 and are proceeding to test for virulence phenotypes in Arabidopsis, tomato, tobacco and soybean. Mutations in the Orf4 protease domain have been generated and are being tested in bean. We hypothesize that these knockouts will cause a reduction in virulence on one or more host plants. If correct, we will then work to identify the targets of these proteases, thus providing insight into the molecular basis of disease susceptibility in plants.

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1. Shao, F., P.M.Merritt, Z. Bao, R.W. Innes, and J.E. Dixon. 2002. A *Yersinia* effector and a *Pseudomonas* avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis. Cell. 109: 575-588.

492 Lipid Signaling in Plant Defense

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Lipid derived second messengers participate in signal transduction mechanisms influencing plant growth, development and response to abiotic and biotic stresses. Polyunsaturated fatty acids (PUFA) released from membrane lipids are precursors for the synthesis of oxidized lipids (oxylipins), some of which are potent signal molecules. For example, jasmonic acid (JA), which is synthesized from linolenic acid (18:3) by the octadecanoid pathway, participates in plant development and stress responses. In addition, a parallel hexadecanoid pathway in Arabidopsis thaliana synthesizes other oxylipins from hexadecatrienoic acid (16:3). We had previously shown that loss of a stearoyl-ACP desaturase activity encoded by the Arabidopsis SSI2 (FAB2) gene confers enhanced resistance to the bacterial pathogen Pseudomonas syringae, the oomycete pathogen Peronospora parasitica. In contrast, resistance to the necrotrophic fungal pathogen, Botrytis cinerea, is depressed in the ssi2 mutant plant. In addition, loss of SSI2 activity also alters plant growth and development; the ssi2 mutant plant is stunted and spontaneously develops lesions containing dead cells. The ssi2 mutant is blocked in the desaturation of stearic acid (18:0) to oleic acid (18:1), resulting in increased accumulation of 18:0 and lowered levels of 18:1. Second site mutations that suppress the ssi2 phenotypes have been identified. Characterization of four complementation groups of suppressor mutants (sfd) indicates that the high level of 18:0 does not have a causal role in the ssi2 phenotypes. Instead, all four groups of suppressor mutations have altered levels/distribution of hexadecatrienoic acid (16:3). ESI-MS/MS-based lipid profiling is underway to study the impact of the ssi2 and sfd mutations on membrane lipid composition and to decipher the contribution of these changes to plant defense responses. Progress on the cloning of the sfd1 will also be presented.

493 The AvrPphE family of type III effectors from diverse phytopathogenic bacteria encode enzymes from the tranglutaminase superfamily

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Many plant pathogenic bacteria employ the type III secretion system in order to inject effector proteins directly into the host cell during infection. On susceptible hosts, these effectors contribute to pathogenesis most likely by interacting with and manipulating host defense and signaling pathways. On resistant plants, some effectors activate specific host Resistance proteins (R protein). Biochemical functions for most effectors cannot be deduced from their primary sequence and it is not clear how they function during pathogenesis. AvrPphE is a type III effector that is widespread among different *Pseudomonas syringae* strains, and even other plant pathogenic bacteria like *Xanthomonas*and *Ralstonia*. We determined that AvrPphE-like proteins are members of the transglutaminase super family, which encode enzymes possessing a catalytic triad of cysteine, histidine and aspartic acids. This catalytic triad is conserved in AvrPphE family members and these residues are required for R-specific recognition on resistant bean hosts. In addition, AvrPphE induces a cytotoxic response on Arabidopsis that is also dependent on the catalytic triad residues. This function may represent a virulence activity for AvrPphE family members. Using a substrate-trapping assay we have identified potential host targets that interact with AvrPphE in planta. Evidence for a genetic and biochemical role for these interactors in AvrPphE function will be presented. Also we will describe use of Arabidopsis to identify mutants defective in AvrPphE-induced cell death.

494 *PBS3* is a Member of the *JAR1* Acyl-Adenylase Family and Regulates Salicylic Acid Levels in Arabidopsis

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The *pbs3* mutant shows enhanced susceptibility to *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000 strains that carry *avrPphB, avrRpt2, avrB* or *avrRps4*. In all cases, however, the level of bacterial growth is intermediate between a fully resistant and a fully susceptible interaction. In addition, *pbs3* mutant plants display enhanced susceptibility to a normally virulent DC3000 strain containing no added avirulence genes. These data are similar to those obtained with transgenic Arabidopsis plants that express *NahG*, a bacterial gene that encodes salicylate hydroxylase. Therefore, we analyzed SA levels in *pbs3* plants inoculated with *Pst*DC3000. SA levels in the *pbs3* mutant were significantly lower than in Col-0 wild-type plants. Consistent with this finding, induction of PR1 gene expression by *Pst*DC3000 (*avrRpt2*) in the *pbs3* mutant was strongly attenuated.

The *PBS3* gene has been mapped to chromosome 5 between the SSLP (simple sequence length polymorphism) markers nga249 and nga151. Through high resolution mapping, we narrowed the position of the *PBS3* gene to a 300-kb interval. We obtained T-DNA insertion lines for approximately half of the predicted genes in this interval, and found one line that displayed an enhanced susceptibility phenotype similar to *pbs3*. The gene disrupted by the T-DNA in this line, *At5g13320*, was sequenced from the *pbs3* mutant, and found to contain two point mutations. *At5g13320* is similar to the auxin-induced soybean gene *GH3*, and also has high similarity to 18 other Arabidopsis genes including the *JAR1* (jasmonic acid responsive 1) gene. *JAR1* is one of the key genes in the Jasmonic Acid (JA) response pathway, which is also a part of the plants' defense machinery. JAR1 belongs to the acyl adenylate-forming firefly luciferase superfamily, and it has been shown that JAR1 adenylates JA (Staswick et. al. Plant Cell 2002). We are currently pursuing biochemical analyses on the PBS3 protein. A possible model linking acyl-adenylase activity to regulation of SA levels will be presented.

495 Complementation of disease resistance mutants by heterologous R genes

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The *Rpg1b* and *RPM1* NBS-LRR disease resistance genes are found in soybean and *Arabidopsis*, respectively. Each of these genes confers the ability to recognize the *P. syringae* pathogen molecule AvrB, and thus mediates disease resistance in the host plant. Although *Rpg1b* and *RPM1* share the same recognition specificity for AvrB, they share limited sequence homology, and recent phylogenetic analysis in our lab suggests that these two genes are not orthologous, but may have acquired the AvrB specificity through convergent evolution. Recent cloning of *Rpg1b* has allowed us to investigate whether molecular recognition of AvrB in soybean is achieved using the same mechanisms employed by *Arabidopsis*. If *RPM1* and *Rpg1b* can provide AvrB recognition in soybean and *Arabidopsis* respectively, this would strongly suggest that these two plant lineages have evolved the same mechanism for activating defense responses upon recognition of AvrB. Experiments are currently underway to determine whether the soybean *Rpg1b* gene can complement an *Arabidopsis rpm1* mutant, and vice versa. To analyze Rpg1b function, an *Arabidopsis rps3-3 (rpm1)* line will be stably transformed with *Rpg1b* under the control of its own promoter, and growth of *Pseudomonas* strains expressing AvrB will be measured in this transgenic line. To examine RPM1 function in soybean, *RPM1* under the control of its own promoter will be transiently expressed in soybean *rpg1b* leaves using a dual-luciferase microprojectile bombardment assay recently developed for soybean. Activity of RPM1 will be measured by cell death resulting from the AvrB/R protein interaction, and subsequent reduction of reporter gene expression. If functional complementation is observed in either of these assays, this would suggest that Rpg1b and RPM1 may utilize the same strategies to recognize the AvrB effector molecule and/or initiate downstream defense responses.

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496 Genetic and genomic approaches towards understanding plant response to the phloem feeding aphids

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Insects that feed on plants can be broadly classified into the chewing insects and the sucking insects. The Phloem feeders/sap suckers represent a unique class of sucking insects, which differ from others in their mode of feeding and in their metabolic ability to detoxify plant defense compounds. These insects cause extensive damage to plants. In addition, phloem feeders like aphids are carriers for economically important plant viruses. However, very little is known about plant defense mechanisms to these phloem feeders/sap suckers.

We have established a model system consisting of the plant, *Arabidopsis thaliana* and the phloem feeder, Green peach aphid (GPA; *Myzus persicae*) to study plant response to feeding by aphids. This system offers the combined power of genetics, molecular biology and metabolomics to enhance our understanding of plant response to aphids. Mutant screens have identified two Arabidopsis mutants, which exhibit altered levels of resistance to GPA. Proteins encoded by both these genes are involved in fatty acid metabolism and in plant response to pathogens. Electrospary-ionisation tandem mass spectrometry (ESI/MS-MS) technology has been used to profile changes in plant lipid composition, in these mutants. A parallel genomics approach to gain insights into the changes in plant genome expression (approximately 23,000 genes) in response to aphid feeding has been accomplished using the Arabidopsis whole genome microarray chip (Affimetrix ATH1). These differentially expressed genes fall into various functional classes, for example, carbohydrate metabolism, photosynthesis, transcription factors, signal transduction, and those involved in defense related mechanisms .A reverse genetics approach is being employed to study the role of these differentially expressed genes in plant response to aphid feeding. Identification of plant genes involved in resistance/susceptibility to aphids will provide us with necessary molecular tools to better understand plant defenses against aphids, and furthermore, engineer/breed aphid resistance plants.

497 Elicitors of Arabidopsis defense responses in the bacterial wilt pathogen, *Ralstonia solanacearum*

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Plants can recognize the presence of bacterial flagellins and induce defense responses. In Arabidopsis, recognition of a conserved N-terminal domain of flagellin is mediated by the transmembrane leucine-rich repeat receptor-like kinase FLS2. Because plants recognize motifs from other bacterial flagellins, we tested the possibility that flagellin from the phytopathogen *R. solanacearum* would elicit plant defenses. Flagellin protein is present in crude boiled bacterial extracts and extracts from wild-type *R. solanacearum* has defense-eliciting activity. However, extracts from flagellin-less mutants were also active. These results indicate that flagellin is not a major elicitor of plant defenses in *R. solanacearum* and that an additional elicitor must be present. We found that this novel elicitor is a protein larger than 10 kDa whose production is not affected by mutations in *PhcA, PehSR, HrpB, or GspM* - all known regulators of virulence. Since an oligopeptide derived from a conserved region of *R. solanacearum* flagellin is not recognized by Arabidopsis, this pathogen may elude host detection by producing a flagellar protein with alterations in the conserved N-terminus.

498 Characterization of genes induced by the Pto resistance pathway

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Pto confers specific resistance to *Pseudomonas syringae* pv. *tomato* expressing AvrPto. Overexpression of Pto in tomato also induced general disease resistance. We have isolated a large number of cDNA from tomato that are induced by Pto overexpression and a large number of cDNA from tobacco that are induced by AvrPto-mediated gene-for-gene resistance. Among them, one 1_F12 encodes a NBS-LRR protein, 5_E08 encodes an Rcr3-like cystein protease, BG352048 encodes an adenylate translocator, and 5_F10 encodes a polyubiquitin like protein. These genes appear to be signal transduction components that may have an important function in plant disease resistance. To test whether these genes have a general role in disease resistance, we applied reverse genetics approach to study their cognate genes in Arabidopsis. 1_F12, 5_E08, BG352048 and 5_F10 are 74%, 72%, 69%, and 77% homologous to the Arabidopsis At3g50950, At3g49340, At3g08580, and At4g12570, respectively. Northern and microarrays analysis indicated that A3g50950, and At3g08580 are induced in Arabidopsis by inoculation with *P. s.* pv. *maculicola* with or without AvrB. The expression of At3g50950 gene is affected by mutation in the SA, JA, and ethylene signal transduction. The expression of At3g08580 is affected by mutation in ethylene transduction pathway. Homozygous T-DNA knockout lines have been identified for At3g50950, At3g49340, and At4g12570. These mutant plants did not show defects in resistance to virulent pathogen P. s. pv. maculicola and avirulent pathogen *P. s.* pv *maculicola* carrying AvrB. We were unable to identify a homozygous T-DNA knockout line for the At3g08580 gene in three generations despite heterozygous T-DNA knockout plants were isolated, suggesting that mutation of this gene may be lethal.

499 Capillary Electrophoresis-Based Profiling and Quantitation of Salicylic Acid and Related Phenolics for Analysis of Early Signaling in Arabidopsis Disease Resistance

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A capillary electrophoresis-based method was developed for quantitation of total salicylic acid levels in Arabidopsis leaves. Direct comparison to previous HPLC-based measurements showed similar levels of salicylic acid. Simultaneous quantitation of *trans*-cinnamic acid, benzoic acid, sinapic acid and an internal recovery standard was achieved. A rapid, streamlined protocol is presented where requirement for plant tissue was markedly reduced relative to HPLC-based protocols. Complicated, multiparameter experiments were thus possible despite the labor-intensive nature of inoculating plants with bacterial pathogens. As an example of this sort of experiment, detailed time course studies were performed of total salicylic acid accumulation by wild type Arabidopsis and two lines with mutations affecting salicylic acid accumulation in response to either of two avirulent bacterial strains. Accumulation in the first twelve hours was biphasic. The biphasic nature of plots of total salicylic acid provides strong evidence for a salicylic acid degradation pathway. The first phase was partially *SID2* and *NDR1*-dependent with both bacterial strains. The second phase was largely independent of both genes with bacteria carrying *avrB*, but dependent upon both genes with bacteria carrying *avrRpt2*. Virulent bacteria did not elicit salicylic acid accumulation at these time points. Longer time points showed very complicated patterns of salicylic acid accumulation. These complications could easily have led to serious misinterpretations of signaling pathways unless combined with the detailed early time course data. Application of this method to various Arabidopsis pathosystems and the wealth of available disease resistance signaling mutants will refine knowledge of disease resistance and associated signal transduction.

500 The *pen* mutants exhibit increased penetration to a non-host pathogen

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Plants are constantly exposed to a wide variety of pathogens. However, a given plant species is host to only a subset of these pathogens. Resistance to pathogens outside this subset is termed non-host resistance. Non-host resistance is thought to be multigenic, non-specific, and durable. In contrast, the resistance of certain genotypes of an otherwise susceptible species to a pathogen is termed host resistance. This type of resistance often follows gene for gene interactions and is typically short lived in the field. Isolating plant factors that affect non-host resistance may lead to a better understanding of non-host resistance. *Arabidopsis* is a host to the powdery mildew *Erisyphe cichoracearum*, and a non-host to *Blumeria graminis* f.sp. *hordei*, a pathogen of barley. A cytological comparison of host and non-host resistance showed that non-host resistance occurred early (1dpi) and correlated with the formation of papillae and strong callose accumulation. While most spores were arrested at penetration, 6% of non-host spores formed haustoria. 12,000 EMS-mutagenized plants were screened for increased penetration of the non-host mildew on *Arabidopsis*. Three different loci were identified. Two of these were allelic to *pen1* and *pen2*, penetration mutants isolated simultaneously in other labs (Hans Thordal-Christensen and Paul Shulze-Lefert, personal communication). The third locus, *pen3*, is unique in that it permits both increased penetration by the non-host fungus and increased hyphal growth. Both *pen2* and *pen3* are more susceptible to *Phytophthora infestans*, another non-host pathogen.

501 Transgenic cell lines: Tools to ID targets of pathogen effector proteins

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It is speculated that resistance gene (R-Gene) products function as receptors for protein ligands produced directly or indirectly by pathogen avirulence (avr) proteins. Previous work has shown that the *Arabidopsis RPS5* gene mediates resistance against the pathogen *Pseudomonas syringae* carrying the avirulence gene *avrPphB*. We have recently determined that AvrPphB functions as a cysteine protease (1), and that one substrate of AvrPphB is the *Arabidopsis* PBS1 kinase (see poster by C. Golstein and J. Ade). We believe that there are additional substrates of AvrPphB in *Arabidopsis*, however, as AvrPphB suppresses defense responses in *Arabidopsis* lines lacking PBS1. As one approach to identifying additional substrates, we have created stably transformed *Arabidopsis* cell lines carrying a dexamethasone-inducible *avrPphB* gene. Dexamethasone inducible constructs containing *avrPphB* and a protease inactive form, *C98S*, were transformed using *Agrobacterium* into both Landsberg and Columbia *Arabidopsis* cell lines. Stable lines were selected and protein extracted after Dexamethasone induction. Western analysis was used to verify the presence of transgenic proteins, and loss of PBS1 in the presence of wild-type AvrPphB, but not protease-inactive AvrPphB. These lines will be now used for proteomic analyses to identify proteins that disappear in an AvrPphB-dependent manner.

502 EDR1, A CTR1-like kinase that regulates defense and stress responses in Arabidopsis

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We have previously isolated the EDR1 (enhanced resistance 1) gene in Arabidopsis thaliana. The edr1 mutant exhibits increased resistance to powdery mildew (Erysiphe cichoracearum) compared to wild-type Col-0 plants. EDR1 encodes a CTR1-like kinase, and functions as a negative regulator of disease resistance. Arabidopsis edr1 mutants display spontaneous lesions in the absence of pathogens in some stress conditions, and also senesce more rapidly than wild-type plants in response to ethylene. These phenotypes suggest that EDR1 is also involved in stress response signaling and cell death regulation. The edr1-mediated resistance is dependent on SA-induced defense responses, and is independent of JA and ethylene induced defense responses. The EDR1 protein consists a kinase domain in the carboxy-terminal third and a nonkinase putative regulatory region in the amino-terminal two-thirds. The EDR1 carboxy-terminal protein displays autophosphorylation activity and can phosphorylate the common MAP kinase substrate myelin basic protein in vitro. The EDR1 kinase domain also phosphorylates a kinase deficient EDR1 protein, indicating that EDR1 autophosphorylation can occur via an intermolecular mechanism. Overexpression of a kinase deficient full length EDR1 gene (35S::dnEDR1) in Col-0 wild-type plants caused a dominant negative phenotype, conferring resistance to powdery mildew (Erysiphe cichoracearum), and enhancing ethylene-induced senescence. RNA gel blot analyses showed that 35S::dnEDR1 transgene was highly transcribed in transgenic plants. Western blot analysis, however, revealed that neither the wild-type nor mutant EDR1 protein could be detected in these lines, suggesting that the dominant negative phenotype was caused by translational inhibition rather than by a protein level effect. Interestingly, overexpression of the EDR1 kinase domain alone in Col-0 wild-type plants also causes a dominant negative phenotype. EDR1 is well conserved among both dicots and monocots. The dominant negative effect of the kinase deficient EDR1 may provide a novel strategy for controlling powdery mildew in crop plants.

1. Frye CA, Tang D and Innes RW (2001). PNAS 98, 373-378
2. Tang D and Innes RW (2002). The Plant Journal 32, 975-983
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503 *RPP27* is a *Cf*-like gene in Arabidopsis that confers downy mildew resistance to several isolates of *Peronospora parasitica*

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RPP27 was unexpectedly revealed from genetic analysis of a cross between an "enhanced susceptibility" mutant of Columbia (Col-*edm1*) and Landsberg *erecta*. The mutation was determined to be a 35kb deletion of seven genes that includes the defense regulator *AtSGT1b*, using the *Peronospora* isolate Hiks1 to assess the phenotype of recombinants (Tor et al. Plant Cell 14: 993-1003). We expected a 3:1 F_2 segregation of resistance to susceptible (associated with inheritance of the *sgt1b* deletion), because both accessions share a Hiks1-recognition gene (*RPP7*) that is SGT1b-dependent. We instead observed a 15:1 segregation. The simplest explanation was that Hiks1 is recognized by another R-gene in Ler-0 that is functionally independent of SGT1b. The second gene (*RPP27*) was mapped to a four BAC interval, a candidate R-like gene within this interval was PCR amplified from Ler-0, and this candidate was cloned into vector pCAMBIA3300. Col-*rpp7* plants (Hiks1-susceptible) were transformed with the candidate, and progeny from BASTA resistant selections conferred resistance to Hiks1 and at least three other Col-compatible *Peronospora* isolates. A full-length cDNA was isolated and the deduced amino acid sequences indicated that *RPP27* encodes a protein with a transmembrane spanning region and an extracellular leucine rich repeat domain, structurally similar to *Cf* resistance genes in tomato.

504 Development of Bioinformatics Tools in the Study of Pathogenic Pseudomonads

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High-throughput sequencing plays an ever-expanding role in genetic research. Advancements in sequencing technology stemming from genomic sequencing projects have made feasible their use for analyzing the results of genetic screens, and for mutant library characterization. Such projects can produce tens of thousands of sequences. This volume of raw data precludes comprehensive analysis without the assistance of computational tools tailored to that analysis.

Researchers require tools to automate the processing of raw sequence files, and assemblies. Tools currently exist for performing base-calling, assembly, BLAST analysis, gene prediction and searches for promoter motifs, but there remains a need to develop tools to correlate the data thus derived and present them in a useful form with a minimum of effort from the researcher.

We are currently developing such tools. The *Pseudomonas aeruginosa* PA14 Transposon Insertion Mutant Library, the PA14 Sequencing project database and *Pseudommonas syringae* Type III Effector databases, all presently under development, have surprisingly similar computational and database requirements. We present here representative workflow and database schemes as an approach for fulfilling these requirements.

505 Large-Scale Functional Characterization of Pseudomonas syringae Effectors

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Pseudomonas syringae causes disease by secreting virulence factors, called effectors, into the apoplast or directly into the plant host cell using a TTSS (Type III Secretion System). We have previously identified 13 effectors in *P. syringae* pv. *maculicola* ES4326 and predicted 38 effectors in the genome sequence of *P. syringae* pv. *tomato* DC3000. Only three of the predicted effectors still need to be confirmed. We have now established a series of cloning vectors based on the Gateway system (Invitrogen) to express effectors in *P. syringae* and plants. These vectors allow expression of HA-tagged effectors from their native promoters or the constitutive *npt2* promoter in *P. syringae* and from the 35S promoter and a DEX inducible promoter in plants. Additionally, we have developed Gateway vectors to express putative effectors as fusions to the HR-eliciting AvrRpt2$^{101-255}$ moiety and fusions to UidA in *P. syringae* to study their translocation and expression respectively *in vivo*. We have also started a large-scale cloning project of all confirmed and predicted *P. syringae* effectors and of effectors from related plant pathogens to study their interaction with *Arabidopsis thaliana* ecotypes and with crop plants using the above described vectors. We are particularly interested in surveying effectors for avirulence activities, i.e. determine how widely effectors are recognized by different plant species and ecotypes. We are also surveying effectors for virulence functions with a focus on those activities that modify plant defenses. Finally, we are using these tools to localize effectors within plant cells.

506 Chitin Signaling in Arabidopsis

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Chitin is a polysaccharide composed of β-1-4-linked N-acetylglucosamine. Chitin oligosaccharides are released from fungal cell walls as a result of hydrolytic enzymes and act as potent elicitors of plant defense responses. To investigate the pathways involved in plant chitin perception, Arabidopsis thaliana seedlings were treated with chitin. RNA extracted from these plants was used to analyze transcript abundance using DNA microarrays. Initial analyses of the microarray data and other data identified interesting genes encoding receptor-like kinases, mitogen-activated protein kinases, WRKY transcription factors, and defense-related genes, etc. T-DNA knockout lines corresponding to some of these genes were obtained to further investigate their potential roles in chitin signaling. Collectively, these initial results revealed the possible existence of an independent chitin signaling pathway in Arabidopsis and identified some of the signaling components involved. Our experience indicates that Arabidopsis will serve as an excellent model system to explore the plant response to chitin.

507 Characterization of the direct target genes of NPR1

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Plants employ a variety of strategies to resist pathogen invasion. In addition to specific resistance mediated by R genes, systemic acquired resistance (SAR) can be induced by a local infection, which confers broad-spectrum resistance. The *NPR1* gene is the master regulator of SAR. In an effort to understand the function of this important protein, we identified direct transcriptional target genes of NPR1 by GeneChip® analysis using *Arabidopsis* carrying an *NPR1-glucocorticoid receptor* transgene. Among the genes induced by NPR1, one group encodes proteins involved in processing newly-synthesized polypeptides in the endoplasmic reticulum. These "ER genes" may be required for the secretion of antimicrobial PR proteins during SAR. In support of this hypothesis, plants carrying T-DNA insertions in the ER genes show a decrease in PR-1 secretion and impaired SAR. Furthermore, one of the mutants is hyper-sensitive to chemical inducers of SAR, possibly due to triggering of the unfolded protein response by accumulation of intracelluar PR proteins. As a clue to the common regulation of these ER genes, a novel *cis*-element was identified among the promoter regions of the ER genes.

508 Molecular Genetic Dissection of the RPP7 Resistance Pathway

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The RPP7 gene activates race-specific resistance to the downy mildew pathogen Peronospora parasitica. Previously published genetic epistasis tests have established that RPP7 activates defense response through a signaling mechanism that does not require accumulation of salicylic acid (SA), or components of the ethylene and jasmonic acid response pathways encoded by EIN2, JAR1, or COI1. Furthermore, RPP7 is not suppressed by mutations in a variety of putative signal transducers that are required by various NBS-LRR resistance genes (e.g. pad4-1, ndr1-1, npr1-1, pbs2-1). In an effort to better understand the genetic requirements for signal transduction from RPP7, we have constructed a series of double mutants to test for additive or functionally redundant contributions by known defense signaling components. Most of these combinations support an enhanced level of asexual sporulation. Staining with DAB and Trypan Blue revealed that all of the double mutants are capable of inducing the oxidative burst, but both responses are delayed by days. These results reveal "cryptic" roles for PAD4, PBS2 (a.k.a. RAR1), NDR1, and NPR1 in RPP7 signaling, and suggest that RPP7 activates resistance through multiple signaling pathways that collectively regulate the kinetics of the HR. We have also cloned the RPP7 gene, using map-based methods. This gene belongs to a cluster of eight related CC-NBS-LRR genes on Chr.1, which share significant similarity with RPP8. The RPP7 gene is at least 19 Kb long. Most of the gene is comprised of long introns within short 5' and 3' UTRs. The RPP7 transcript is alternatively spliced. The functional significance of this alternative splicing is currently unknown.

509 Mechanism of CBF mediated plant COR gene transcriptional activation

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Low temperatures play a major determining factor in defining the geographical regions in which any given plant can successfully be grown. Yet plants have adopted strategies that allow them to survive in most geographical regions. One such strategy is cold acclimation, the biological phenomenon in which plants increase their maximal level of freezing tolerance subsequent to a period of low, non-freezing temperatures. Integral to the cold acclimation process are the CBF family of transcriptional activator proteins. The CBFs act by binding to a conserved DNA sequence motif known as the C-Repeat/dehydration responsive element present in the upstream 5-prime promoter region of the Arabidopsis cold regulated (COR) genes, causing the high level accumulation of COR gene transcripts. In turn the encoded COR polypeptides increase the structural integrity of the plant cell helping to protect it from the deleterious effects caused by freeze-induced cellular dehydration. However the mechanisms by which the CBFs stimulate COR gene expression are essentially unknown. As such, one of our goals is to develop a better understanding of the mechanisms and processes the CBF transcriptional activator proteins utilize to stimulate gene expression. Towards this goal we have begun to identify the critical residues of CBF1 required for functional activity in plants through mutagenesis and transgenesis, and the candidate target factors facilitating CBF mediated COR gene activation.

510 A cuticle developmental gene negatively regulates bacterial TTSS expression

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Many gram-negative bacterial pathogens use the type III secretion system (TTSS) to deliver a repertoire of effector proteins into their host cells for parasitism. These bacteria sense host-derived signals to coordinately activate genes encoding TTSS and effectors, collectively called TTSS genes, thus setting off an array of cellular activities in both bacterial and host cells that culminate in a successful infection. However, host signals and bacterial mechanisms that activate these genes are poorly understood. We have developed a highly sensitive reporter assay for Pseudomonas syringae TTSS gene expression in Arabidopsis. The firefly luciferase gene was fused downstream of TTSS gene promoters in *P. syringae*. The TTSS gene expression in planta can then be monitored in real time by placing the infected plant under a low light CCD camera. As a first step to understand the nature of the plant signal(s), we have identified a genetic locus in Arabidopsis that affects TTSS gene expression. The mutant plant supported a ?super induction? of both the TTSS regulator gene hrpL and the effector gene *avrPto* that is a target of HrpL. The mutant, tentatively named *att1* (for aberrant induction of type three genes), may be genetically altered in a major plant signal that regulates bacterial TTSS genes. Molecular cloning of the *ATT1* gene and electron microscopy studies suggest that *ATT1* is required for the development of cuticle structure. The implication of these findings will be discussed.

511 Regulation of Cell Death by *BON1* and *BON3*

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BON1 and its two homologs *BON2* and *BON3* encode calcium-dependent, phospholipid-binding proteins belonging to an evolutionarily conserved copine family. The biological function of this gene family was first revealed by the *BON1* loss-of-function mutant. *bon1-1* has greatly reduced cell size and thus is dwarf at 22!?C but not 28!?C. It also occasionally undergoes precocious cell death at or near the margins of the leaves. In addition, *bon1-1* mutant exhibits constitutive systemic acquired resistance (SAR) including an accumulation of salicylic acid (SA), upregulation of pathogenesis related genes, and enhanced resistance to virulent pathogens *Pseudomonus syringae* and *Peronospora parasitica*. *bon1-1* phenotype can be suppressed by expressing the SA- degrading enzyme nahG, suggesting that the phenotypes of *bon1* mutant are SA dependent. We hypothesize that *BON1* plays a critical role in maintaining cellular homeostasis, and the loss of its function leads to cell death, activation of plant defense response, and reduced cell growth.

A role of *BON1* in cell death control is further revealed by *bon1;bon3* double mutant analysis. Overexpression of *BON3* complements the *bon1-1* mutant, indicating a conserved biochemical activity between the two genes. The *BON3* loss-of-function mutant does not show any abnormal phenotype. However, *bon1;bon3* double mutant has arrested growth at an early developmental stage and eventually dies at 22!?C. It is likely that the arrested growth is due to cell death at the shoot apex, and *BON1* and *BON3* have overlapping functions in maintaining cellular homeostasis and inhibiting cell death.

512 Three Feedback Loops and Nitric Oxide Production Control the Arabidopsis Hypersensitive Response

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In order to understand how components implicated in the plant hypersensitive response (HR) function together as an integrated system, *Arabidopsis thaliana* HR progression and associated signaling were measured using mutants affecting feedback loops. In response to *Pseudomonas syringae* pv. tomato DC3000&middot:*avrB*, *npr1-2* plants showed increased cell death. Hydrogen peroxide accumulation was similar to wild type. Salicylic acid (SA) accumulation was reduced 6-7 hours post-inoculation. With DC3000&middot:*avrRpt2*, similar trends were seen except that impairment in SA accumulation came 4-5 hours post-inoculation. With DC3000&middot:*avrB*, *ndr1-1* plants showed increased cell death relative to wild type or *npr1-2* plants. Hydrogen peroxide accumulation was delayed and reached half the level seen with wild type plants. SA accumulation was impaired 5-8 hours post-inoculation. With DC3000&middot:*avrRpt2*, *ndr1-1* plants showed no cell death, no hydrogen peroxide accumulation and minimal SA accumulation. Results with a *ndr1-1/npr1-2* double mutant were similar to *ndr1-1*. These data support HR control by one positive and two negative feedback loops but argue against direct negative autoregulation of SA biosynthesis. Kinetics of NO production were followed by measurement of green DAF-FM T fluorescence in leaves co-infiltrated with DAF-FM diacetate. Kinetics of hypersensitive cell death were followed by red fluorescence of co-infiltrated propidium iodide. Neither NO accumulation nor cell death were seen until 3 h post-inoculation of Columbia leaves with DC3000&middot:*avrB* or 5.5 h post-inoculation with DC3000&middot:*avrRpt2*. Subsequent NO accumulation kinetics closely paralleled HR progression in both Columbia and *ndr1-1* mutant plants. These data established that NO accumulation does not happen sufficiently early for NO to be a signaling component controlling HR triggering. NO accumulation did contribute to the HR, as proven by about 1 h delay in cell death kinetics caused by a NO scavenger or a nitric oxide synthase inhibitor. NO was first seen at punctate foci on the cell surface. Subsequent NO accumulation patterns were consistent with NO being an intercellular signal that functions in cell-to-cell spread of the HR.

513 RPS4-mediated disease resistance requires the combined presence of multiple *RPS4* transcripts

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Arabidopsis *RPS4*, a disease resistance (*R*) gene belonging to the Toll/interleukin-1 receptor (TIR)-nucleotide binding site (NBS)-leucine-rich repeat (LRR) class of *R* genes, confers resistance to *Pseudomonas syringae* p.v. tomato DC3000 expressing *avrRps4* in a gene-for-gene specific manner. Like other family members in different plant species, *RPS4* produces alternative transcripts with truncated open reading frames. The dominant alternative *RPS4* transcripts are generated by intron retention of intron 3 or introns 2 and 3, which contain in-frame stop codons and lie downstream of the NBS-encoding exon. Here we analyzed the biological significance of these alternative transcripts. We removed introns 2 and 3, either singly or in combination, from the functional *RPS4-Ler* allele and assayed for function of the altered genes by transformation of the naturally susceptible accession RLD. Removal of one or both introns abolished function of the *RPS4* transgene, while expression was not affected. In addition, a truncated *RPS4-Ler* transgene encoding the putative TIR and NBS domains was not sufficient to confer resistance, suggesting that the combined presence of multiple *RPS4* transcripts is necessary for complete function. Interestingly, we observed partial resistance in a subset of transgenic lines expressing both an intron-less and a truncated transgene, confirming the requirement for multiple transcripts for *RPS4* function. Since the truncated transgene transcripts do not resemble naturally occurring alternative transcript, this may indicate that alternative transcripts function on a protein level rather than as regulatory RNAs. Together with results on the tobacco *N* gene, our data suggest that the generation of alternative TIR-NBS-LRR *R* gene transcripts is of general biological significance across plant species.

514 mos mutants affecting NPR1-independent disease resistance in snc1

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Plants have evolved sophisticated defense mechanisms against pathogen infections, during which resistance (R) genes play a central role in recognizing pathogens and initiating defense cascades. In Arabidopsis, a dominant mutant, snc1, was previously identified that constitutively expresses pathogenesis-related (PR) genes and resistance against both Pseudomonas syringae maculicola ES4326 and Peronospora parasitica Noco2 in the npr1-1 background. We have confirmed that SNC1 encodes an RPP5 homolog and a single amino acid change in the region between the NB-ARC and LRR of SNC1 renders this R-protein constitutively active. To identify genes important for the NPR1-independent pathogen resistance, we carried out a genetic screen to identify mos (modifier of snc1) mutants that affect the NPR1-independent PR gene expression in the snc1 npr1 plants. About 50 mos mutants have been isolated. Mapping and complementation analysis showed that these mutants represent a minimal of 6 complementation groups. In mos1, both the constitutive PR gene expression and pathogen resistance have been abolished. The mutation in mos1 is mapped to a small region on chromosome 4. We are currently sequencing this region to identify the mutation in mos1. Characterization and cloning of mos1 will be presented.

515 Charaterization of RPS4 and AvrRPS4

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RPS4 is a disease-resistance locus on chromosome 5 of *Arabidopsis thaliana* , which confers specific resistance to *Pseudomonas syringae pv. tomato* carrying the avirulence gene *AvrRPS4*. Ler, Col-0 and Ws-0 ecotypes carry a resistant allele, while RLD harbors a susceptible allele. RPS4 belongs to TIR-NBS-LRR R protein family and its disease-resistance signalling is EDS1 dependent. However, the mechanism by which R proteins function to recognize pathogen-derived ligands and activate defence response is unclear. We have used the *RPS4-AvrRPS4* system to try to dissect this mechanism.

Transient overexpression of *RPS4* in tobacco induces *AvrRPS4*-independent hypersensitive response (HR). Deletion analysis of RPS4 domains showed an absolute requirement of the TIR domain for the HR phenotype. A point mutation in the P-loop motif of the NBS domain abolishes the cell death. It suggested the requirement of a functional NBS domain in the HR process. Using virus induced gene silencing (VIGS), it showed that the RPS4 overexpression induced cell death is EDS1 and SGT1 dependent, but not NDR1 dependent.

When transiently delivering C-terminus epitope tagged *AvrRPS4* into plant cell, we found that AvrRPS4 is always processed from the N-terminus. To verify whether the processed AvrRPS4 is still functional, a series of N-terminal deletion constructs of *AvrRPS4* were made. HR test in *Arabidopsis* turned out that the processed form is still functional. This means that N-terminus of AvrRPS4 does not affect its function once the protein is expressed in plant cell directly. *In vitro* secretion assay showed that the process does not occur either inside of *Pst* or outside of *Pst*. In another word, secreted AvrRPS4 from *Pst* is still mature. Therefore, it suggested that the N-terminus process occur inside of plant cell.

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516 Dorsoventral asymmetry in the *Arabidorrhinum thalimajus* flower

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Dorsoventral asymmetry in flowers is thought to have evolved independently multiple times in different species from radially symmetric ancestors. In *Antirrhinum*, dorsoventral asymmetry of the flower and its component organs requires the combined activity of four key genes: *CYCLOIDEA* (*CYC*), *DICHOTOMA* (*DICH*), *RADIALIS* (*RAD*) and *DIVARICATA* (*DIV*). We are currently analysing how these genes, all coding for transcription factors, interact to establish a basic asymmetric pre-pattern in the *Antirrhinum* flower meristem and exploring the extent to which these processes are conserved in *Arabidopsis*, a species with radially symmetric flowers. *CYC*, *DICH* and *RAD* are expressed dorsally in floral primordia and promote dorsal petal and stamen identity. Genetic studies have revealed that *RAD* is downstream of *CYC* and *DICH*. We have obtained the DNA-binding site consensus for CYC protein by random binding-site selection and shown that CYC can bind directly to the *RAD* promoter and intron. In Arabidopsis, the *CYC/DICH* orthologue, *TCP1*, is also expressed asymmetrically in young floral meristems but, in contrast to *CYC*, *TCP1* expression is transient and cannot be detected in floral primordia older than stage 2. Six *RAD* homologues were isolated from *Arabidopsis*; however, no expression was detected in the dorsal region of Arabidopsis floral primordia. Moreover, the expression of *RAD::RAD* in Arabidopsis transgenic plants did not overlap with *TCP1* expression. These results suggest that the recruitment of *RAD* expression in the dorsal domain of *Antirrhinum* floral primordial might have been important for the generating a flower with dorsoventral asymmetry. In addition, we overexpressed *RAD* in *Arabidopsis* and the transgenic plants obtained were dwarfed with smaller epidermal cells.

517 QTL analysis of growth related traits in a new recombinant inbred population derived from the L*er* x Sha cross.

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Arabidopsis natural variation was used to analyze growth rate and related traits to unravel the genetic background of these traits. Screening of 22 accessions revealed a large variation between them for plant dry weight, seed weight and relative growth rate but not for water content. A positive correlation was observed between seed weight and plant area 10 days after planting, suggesting that seed weight affects the plant growth during early phases of development. During later stages of plant growth this correlation was not significant, indicating that other factors determine growth rate. Quantitative trait locus (QTL) analysis, using 114 (F9 generation) recombinant inbred lines (RILs) derived from the cross between L*er* and Shakhdara (Sha), showed QTLs for relative growth rate (RGR), chlorophyll fluorescence, dry weight, fresh weight, seed weight, flowering time and other traits related to flowering. Co-location of some QTLs was observed for plant area and for flowering-related traits, such as leaf number. QTLs for fresh and dry weight co-located with QTLs for plant area, RGR and total leaf number, suggesting that these traits might be controlled by the same gene(s) and therefore might be pleiotropic. At chromosome 5, a QTL for flowering time co-located with a QTL for leaf area & leaf initiation but not for fresh or dry weight, meaning this locus might be involved in speed of leaf initiation. QTLs for a trait like flowering time, that have been analyzed in other RIL populations, sometimes overlapped with known QTLs but also novel loci were identified.

518 Gene Expression Analysis Between Arabidopsis Ecotypes Identifies a Quantitative Trait In Lateral Root Development.

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Naturally occurring variation in gene expression patterns in plants can be the result of adaptive evolution in response to differing environments. Oligonucleotide based arrays are becoming the choice of many researchers in gene expression analysis due to their high specificity. However, polymorphisms between ecotypes can interfere in array hybridization by reducing the signal intensity. To address this issue and to examine natural variation in gene expression patterns, we compared RNA samples from three *Arabidopsis thaliana* ecotypes (Col, Ler and CVI) grown under identical conditions. ANOVA based expression analysis proved to be highly effective, identifying significant changes of less than 1.5 fold in gene expression. Comparison of the top 100 genes having the least different expression patterns, indicated that long oligos could withstand up to 3 bp mismatch without interference in signal intensity. Our genome wide expression analysis identified three groups of genes, one group of genes that are up- or down- regulated exclusively in one particular ecotype in comparison to the other two, the second group of genes corresponds to those for which expression differs only between one pair of ecotypes (e.g., Col-Ler, Col-CVI, or Ler-CVI), and the third group comprises those in which expression differs across all pair-wise combinations. Number of lateral root produced from a primary root is determined by auxin signaling and nutrient availability in the soil. Our genome wide expression analysis identified a differentially expressed gene (LRP1) between ecotypes which is specific to lateral root initiation. Based on this information we have identified a phenotypic variation between Col and Ler. Using recombinant inbred lines, we have mapped the quantitative trait loci controlling the number of lateral roots in Arabidopsis.

519 The power of movement revisited: Assessing the adaptive evolution of phototropism in Arabidopsis

Candace Galen, Julie Huddle, Mannie liscum
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All plants exhibit phototropism, the capacity for directional bending towards blue light (BL). Like the phytochrome-mediated shade avoidance response, phototropism represents a source of morphological plasticity in plant architecture. Yet, unlike shade avoidance, the adaptive significance of phototropism has been largely a topic of conjecture. In Arabidopsis, phototropism of seedlings is under the control of two paralogous BL receptors, phot1 and phot2, whose functions are both partially overlapping and partially unique. In particular, phot1 modulates phototropic responsivess to both low and high fluence rate BL, while phot2 appears to function exclusively as a high fluence rate receptor. The NPH3 protein, which has been shown to physically associate with phot1, represents an early post-perception signaling element in the phototropic signal-response pathway. We tested the fitness of wild-type, *phot1*, *phot2*, and *nph3* mutants over a range of light conditions in the field and found that genotypes varied significantly in fitness at each lifecycle stage. Genotype-by-environment interaction for fitness was significant only during emergence. Environmental effects on fitness were also substantial later in the life cycle, but affected all genotypes equivalently. These results demonstrate an important adaptive value for phototropism under natural conditions. Moreover, our studies demonstrate that the maintenance of a suite of alternative sensory systems, in this case the phot1 and phot2 signal-response pathways, contributes to ecological amplitude.

520 Expression and evolution of the polygalacturonase gene family

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Polygalacturonases(PGs) in Arabidopsis constitute a relatively large gene family that is expressed in numerous tissue types and at different developmental stages during the life cycle of a plant. PGs are highly homologous and are known to regulate most, if not all, of the processes associated with cell separation. Although there have been studies on PG expression, gene disruption, and antisense, these have met with limited success due to high homology within this gene family. We have identified all the PGs from the Genbank protein records including the complete Arabidopsis and rice genomes. PGs are found in a few metazoa, eubacteria and fungi as well as plants. Within the plant kingdom, PGs are found in Gymnosperms, monocots and dicots. Based on the phylogeny of rice and Arabidopsis, we classify the PGs into 3 major clades, 9 subfamilies, and 5 subgroups. Both rice (42) and Arabidopsis (66) PGs are found in all 3 major clades indicating that they originated from a common ancestor of monocots and dicots. To understand more about the potential mechanisms contributing to the expansion of the PG family in Arabidopsis, we examined the distribution of Arabidopsis PGs on all 5 chromosomes. We also determined the expression pattern of all 66 Arabidopsis family members in five different tissue types and five different stages of floral organ abscission using RT-PCR. We have studied the RT-PCR expression patterns in the context of genetic and evolutionary relationship. We will discuss the evolutionary implications of this data and present theories of gene expansion and function.

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521 SNP Genotyping Of *Arabidopsis thaliana* Ecotypes Using Pyrosequencing And Fluorescence Polarization

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Single Nucleotide Polymorphisms (SNP) have the potential for allowing high-throughput genotyping and fine-scale mapping needed for Linkage Disequilibrium studies and for mapping QTLs. These markers will accelerate the identification of genetic determinants of natural phenotypic variation. We have used Pyrosequencing for SNP genotyping and have implemented a low-cost genotyping platform based on single-nucleotide extension and fluorescence polarization (FP) for allele discrimination between ecotypes. We have confirmed the validity of these markers in Col/Ler RI lines and have used these markers to genotype 94 accessions at 0.5-mega base resolution. In addition we have genotyped these accessions with 200 markers on Chromosome III. We have also used Pyrosequencing markers for quantitative estimation of allelic frequencies in a mapping population as a tool for rapidly estimating map positions.

For high-density (> 1,200) SNP maps, it is impractical to employ genotyping methods that allow analysis of only one (TaqMan, Pyrosequencing, FP) or a few (MALDI of extension products) SNPs at a time. We will implement a protocol for genotyping polymorphisms using the ParAllele SNP genotyping platform (Parallele Biosciences, South San Franciso, CA) by means of padlock probes, and identify markers that discriminate between 94 accessions of *Arabidopsis* with a minor allele frequency >10%. SNP genotyping with this method consists of manufacturing pools of thousands of padlock probes, each with terminal regions that target individual SNPs, a barcode tag sequence that will subsequently recognize an oligonucleotide probe array, and a set of common PCR primers. These probes are mixed with genomic DNA, annealed, and separated into 4 identical reactions. The gap-fill ligation reactions are then carried out with a different dNTP in each tube. The samples are then amplified using a single set of common primers and the fragment content of each reaction is assayed using a standard oligonucleotide probe array. Progress on these projects will be presented.

522 EVOLUTIONARY ANALYSIS OF PLANT C-REPEAT/DRE BINDING FACTOR (CBF) FAMILY

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The Plant C-repeat/DRE Binding Factor (CBF) family is a highly conserved component in the plant genome cold-response pathway. We collected 49 CBF sequences from 12 different species in monocots and dicots to examine the molecular evolution of this family. The CBF family diverged at the time of angiosperm diversification about 180-200 million years ago, but there was no clear delineation between ?acclimated? and ?non-acclimated? species in the phylogeny. A reconciled tree between the CBF gene tree and a species tree allowed us to infer gene duplication events. According to our currently collected samples, the CBFs in monocot and dicot clades show different evolutionary patterns. In the dicot clade, gene duplication occurred in two stages: the first stage happened just after monocot and dicot diversification; the second stage happened about 35 million years ago before the diversification of Brassicaceae. Monocot gene duplication occurred in multiple stages about 60-100 million years ago before the radiation of Poaceae. The duplication events in CBF family correspond with previously inferred ancient plant genome duplications.

523 Variability in homogeneous and heterogeneous *Arabidopsis* accessions

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Natural populations of *Arabidopsis thaliana* can exhibit considerable phenotypic variation. Our goal is to characterize natural variability in sensitivity and resistance to multiple stresses such as ozone, CO_2, and temperature related to global climate change. However, because most seed bank accessions are derived from one or a few original samples, then bulk propagated, they may be relatively homogeneous genetically. Some accessions, though, were originally collected from many plants and may be more heterogeneous. We therefore are conducting this preliminary study to determine if heterogeneous accessions exhibit a greater range of variability, perhaps more characteristic of the natural populations. From a study of 30 widespread accessions, seven "homogeneous" and seven "heterogeneous" accessions were selected. For each accession, 60 plants were grown to determine frequency distribution patterns in developmental, growth, and reproductive parameters. Outcrossing among individuals was prevented to determine heritable variation in future studies. In general, phenotypic variation within accessions was less in developmental characters - under stronger genetic control - than variation in growth parameters and reproductive output. Most heterogeneous accessions were significantly more variable than homogeneous ones. Moreover, frequency distributions varied significantly in shape and breadth. Most flowered by 40 days ("early-flowering") and produced <40 leaves at bolting. Late-flowering accessions took up to 120 days to flower and produced as many as 100 leaves. Leaf production rates varied continuously among the 30 accessions, though within an accession, the rates were less variable. Leaf area, estimated as projected leaf area from digital images, was usually the most variable parameter. Preliminary seed analysis showed much less variability in seed size, even among heterogeneous accessions, but significantly different seed production within and among accessions. In summary, heterogeneous accessions do seem to be a source of greater phenotypic variability than homogeneous ones. However, significant differences among heterogeneous accessions suggest differences in the original populations sampled or in the bulk propagation methods.

524 Large-scale identification and analysis of genome-wide single-nucleotide polymorphisms for mapping in *Arabidopsis thaliana*.

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Genetic markers such as single nucleotide polymorphisms (SNPs) are essential tools for positional cloning, association or quantitative trait locus mapping and the determination of genetic relationships between individuals. We identified and characterized a genome-wide set of SNP markers by generating 10,706 expressed sequence tags (ESTs) from cDNA libraries derived from six different accessions, and by analysis of 606 sequence tagged sites (STS) from up to twelve accessions of the model flowering plant *Arabidopsis thaliana*. The cDNA libraries for EST sequencing were made from individuals that were stressed by various means to enrich for transcripts from genes expressed under such conditions. SNPs discovered in these sequences may be useful markers for mapping genes involved in interactions with the biotic and abiotic environment. The STS loci are randomly distributed over the genome. By comparison with the Col-0 genome sequence, we identified a total of 8,051 SNPs and 637 insertion/deletion polymorphisms. Analysis of STS-derived SNPs shows that most SNPs are rare, but that it is possible to identify intermediate frequency framework markers that can be used for genetic mapping in many different combinations of accessions. A substantial proportion of SNPs located in open reading frames caused a change of the encoded amino acid. A comparison of the density of our SNP markers among accessions in both, the EST and STS datasets, revealed that Cvi-0 is the most divergent accession from Col-0 among the twelve accessions studied. All these markers are freely available via the internet.

525 Generation of the Est-1/Col RIL population and QTL analysis of this population for light dependent responses.

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We are utilizing natural variation present in different accessions of *Arabidopsis thaliana* to identify genes involved in light responsiveness. Plants clearly are capable of responding in a variety of ways to specific light cues. We have created a RIL (recombinant inbred line) population between accessions Est-1 (Eastland) and the common lab strain Col (Columbia). We have characterized a population of 280 RILs for light responsiveness by analyzing both hypocotyl elongation and flowering time. QTL (Quantitative Trait Loci) analysis has revealed major QTL in white, blue, red and far-red light conditions for hypocotyl elongation, in addition to flowering time QTL in both short and long days. Two candidate genes for a red light hypocotyl elongation QTL and a long day flowering QTL are *phyB* and *ft*, respectively. Novel QTL affecting light responses were also detected, and all large effect QTL are currently in the process of being confirmed in NILs (Near Isogenic Lines).

526 Quantitative trait loci for trichome density in Arabidopsis thaliana

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Although a good deal is known about the genetics that underlie trichome initiation and development in *Arabidopsis thaliana*, little is understood about loci that affect trichome density in natural populations. Previous quantitative trait locus (QTL) mapping work by others identified at least one locus (Reduced Trichome Number - RTN) with a large effect on trichome density segregating in the Col x Ler recombinant inbred line (RIL) mapping population. However, in a screen of 120 natural accessions, we find more than an order of magnitude range of variation in trichome density that is distributed in such a way as to suggest that many more loci affect this trait. Furthermore, there persists the issue of how general the findings of any one QTL study are in relation to an entire species. To address these issues, we have mapped QTL for trichome density in the Bay x Shah RIL population. Three QTL of significant effect were identified; one of these QTL overlaps positionally with RTN, while the other two appear to be unique to the Bay x Shah mapping population. Mapping results and interpretations will be discussed.

527 Multiplex SNP Genotyping in Arabidopsis using SNPWAVE TM

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Large scale genotyping of genetic markers such as single nucleotide polymorphisms (SNP's) is important to map mutants and identify quantitative trait loci (QTL) in Arabidopsis and other model organisms. In addition, SNP genotyping is essential for marker-assisted selection in plant and animal breeding programs. Since a large diversity of cost-effective platforms for SNP detection exist, the main cost component is preparation of the detection mixture. In order to reduce the cost per datapoint, efficient multiplex amplification technologies and miniaturization of reaction volumes are needed. We are developing a 100-plex SNPWaveTM assay to facilitate whole genome screens and fine-mapping based on SNP genotyping in Arabidopsis. For this purpose, 100 polymorphisms between the Landsberg and Colombia ecotypes were selected from the TAIR database. With 20 polymorphisms per chromosome, the average inter-marker distance ranges between 3 and 6 cM per chromosome. SNPWave is based on highly multiplexed allele-discrimination, followed by selective amplification of 10 SNP loci in a single polymerase chain reaction (PCR) using the amplified fragment length polymorphism (AFLP) technology. The use of AFLP in this context allows the amplification of various subsets of SNP's, including 10 subsets for whole genome screening which each contain 1 SNP of every chromosome arm, or 10 subsets for fine mapping which each contain 10 SNP's derived from a single chromosome arm. Detection of SNPWave reaction products is performed on a (capillary) sequencer, such as the MegaBACETM, using multiple fluorescent labels and short running times. SNPWaveTM allows co-dominant scoring of over 23,000 SNP loci on one MegaBACETM 1000 in a 6 hour period, using 96 capillaries, 4 labels, 10 SNP loci per reaction and 6 consecutive runs. We expect this SNPWave assay to become a valuable and flexible tool for gene mapping in Arabidopsis.

528 The WD-Repeat Protein Superfamily in Arabidopsis

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The WD motif (aka Trp-Asp or WD40 motif) is found in a multitude of eukaryotic proteins involved in a variety of cellular processes. Where studied, repeated WD motifs act as a site for protein-protein interaction, and proteins containing WD repeats are known to serve as platforms for the assembly of protein complexes or mediators of transient interplay among other proteins. The predicted Arabidopsis proteome contains over 250 WD-repeat proteins, approximately one-half of which appear to be plant-specific. The few plant members of this superfamily that have been characterized to date participate in diverse and important events, including regulation of light signaling and photomorphogenesis, seed development, meristem function, epidermal cell patterning, production of anthocyanins, floral development, and timing of flowering. Many yet-uncharacterized members are closely related to well-studied proteins in other eukaryotes with crucial roles in signaling, cytoskeletal dynamics, protein trafficking, nuclear export, RNA processing, transcription, and chromatin dynamics. Defects in specific human WD repeat proteins have been associated with several human diseases, and most of these important proteins are strongly conserved in Arabidopsis. Here I analyze the predicted complement of WD-repeat proteins from Arabidopsis, and compare this to those from budding yeast, fruit fly and humans to illustrate both conservation and divergence in structure and function.

529 Systematical Analysis on Arabidopsis Splicing Related Genes

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Iowa State University

Although splicing machinery is generally conserved among eukaryotic organisms, plant splicing has specific features. In this study, Arabidopsis splicing related genes were computationally identified by BLASTing the Arabidopsis genome using known splicing related genes as query sequences. A database were constructed for these genes and available at http://www.plantgdb.org/AtGDB/prj/SRGD/ASRG-homepage.php . A total of 73 snRNA genes and 262 splicing related proteins were identified. Each major snRNA has 11-18 copies in Arabidopsis and 7-8 of these are active. U2 snRNA has 14-16 active copies. The minor snRNAs, however, tend to have low copy numbers. All active snRNA genes have a conserved USE (Up Sequence Element, RTCCACATCG) and TATA box in promoter regions. Seven U1/U4 clusters and one U2/U5 cluster were found. The 262 splicing related proteins were classified into four groups according to their sequence similarity, exon sizes and domain structures. There were 85 snRNPs (small nuclear ribonucleoproteins), 65 splicing factors, 41 splicing regulators, and 71 unclassified splicing proteins. About 58% of splicing related genes were duplicated. The rate of duplication and the level of variation increase from snRNP to splicing factors and then to splicing regulators. snRNPs are the most conserved, splicing factors are less conserved, and splicing regulators are the least conserved of the three groups, indicating that post-transcription control is becoming more subtle and important than before. It seems that the general splicing mechanism is conserved in plants, but the splicing regulation mechanism is more variable. This is reasonable for allowing organisms to adapt to their environments. Plants may have developed special ways to control splicing during evolution.

530 Stochastic and epigenetic changes of gene expression in *Arabidopsis* polyploids

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Polyploidization is an abrupt speciation mechanism for eukaryotes, especially plants. The fate of redundant genes resulting from genome duplication is poorly understood. Recent studies indicate that both genetic and epigenetic mechanisms are involved in regulating redundant genes of polyploids, which can be either activated or silenced upon polyploidization. However, little is known about the timing and mechanisms of establishing and maintaining differential gene expression. Here we show that in synthetic *Arabidopsis* allotetraploid lines derived from *A. thaliana* and *A. arenosa*, some progenitor's genes are differentially expressed in early generations, whereas others are silenced in late generations or different siblings within a selfing generation, suggesting both rapid and stochastic effects of polyploidization on the expression of progenitor's genes. To test the role of DNA methylation in silencing duplicate genes, we produced DNA-hypomethylation transgenic *A. suecica* lines using RNA interference (RNAi). Some silenced genes were reactivated in both RNAi-*ddm1* and -*met1* lines, consistent with the demethylation of centromeric and rDNA repeats and gene-specific regions in the genome. A rapid and stochastic process of differential gene expression enforced by DNA methylation may contribute to the evolutionary success of polyploids in natural selection and adaptive evolution.

531 Natural Variation of FLM/MAF1

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To discover new loci controlling flowering time in natural populations, we have analyzed the flowering time of a recombinant inbred line (RIL) population derived from crosses of Niederzenz (Nd-1) to Columbia (Col-3 and Col-5; *Holub, et al.*, Adv. Bot. Res. 24[1997]). In short days, the 96 RILs show a wide range of flowering times and QTL analysis detects four significant QTL responsible for this variation: a major QTL, termed *FLOWERING1*, on the bottom of chromosome 1; two QTL on chromosome 2; and one QTL on chromosome 5. *FLOWERING1* maps very close to *FLM/MAF1*, a gene encoding a MADS-domain transcription factor that has been shown by the Amasino and Riechmann labs to have flowering time effects in both long and short days. Additionally, the phenotypes of *flm-1* and *flm-2*, two T-DNA alleles isolated in the Ws background, agree well with that of *FLOWERING1*. Upon attempting to sequence the *FLM* gene from Nd-1, we discovered a 6.8 kb deletion removing the entire coding region. In support of *FLM* being *FLOWERING1*, fine-mapping experiments have located the QTL to a 140 kb interval containing *FLM*. Out of 140 accessions analyzed, only one additional accession from Niederzenz shares this deletion. However, further analysis of the *FLM* genomic region from more accessions revealed significant polymorphism, including transposon insertion events, the nature and consequences of which will be presented.

532 The Swiss-Prot Plant Proteome Annotation Project (PPAP)

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Swiss-Prot is a protein sequence and knowledge database that is recognized worldwide for providing high-quality annotation, minimal redundancy and extensive integration of other relevant databases. Information is gathered and continually updated from the current scientific literature, input from external experts and the application of high-performance bioinformatics tools. By using a standardised nomenclature and controlled vocabularies, Swiss-Prot brings together experimental results, computed features and sometimes even contradictory conclusions. Completion of the genome sequence of model plant organisms has lead us to initiate the Plant Proteome Annotation Project (PPAP) devoted to plant-specific protein families, with a particular emphasis on *Arabidopsis thaliana*. According to ab initio gene prediction, about 26?000 proteins were estimated to compose the *Arabidopsis* proteome. However, automatic gene prediction is prone to errors, and the release of large quantities of full-length cDNA sequences resulted in the improvement by The Institute for Genomic Research of over 35% (more then 9'000) of the predicted gene models. In plants, we are facing large gene families which arise very often by duplication of genes that can differ by only one nucleotide in the open reading frame. In addition, alternative splicing or alternative initiation are not as infrequent as initially thought. Currently, over 2'000 *Arabidopsis* proteins have been curated and integrated into Swiss-Prot, while 41'000 still await manual annotation in TrEMBL. All existing *Arabidopsis* entries are correlated with genomic data and are implicitly linked to the TAIR database. However, *Arabidopsis* data represents only 20% of Swiss-Prot plant entries, the major part concerning about 1'200 others species. In future, the release of additional plant genomes and full-length cDNAs will offer us the opportunity to extend our scope to the annotation of orthologous proteins.

The Internet address of the Swiss-Prot Plant Proteome Annotation Project is: www.expasy.org/sprot/ppap and you can contact us at: PPAP@isb-sib.ch

533 Germplasm Resources for Functional Genomics

Craigon, Daugherty, Gill, Higgins, Humphreys, James, May, Okyere, Schildknecht,Sun
Nottingham Arabidopsis Stock Centre

The Nottingham Arabidopsis Stock Centre (NASC) began in 1991 with just 200 lines. Today, NASC, together with the ABRC, (Arabidopsis Biological Resource Center) each have representation of approximately 400,000 lines, the majority of which are insertion mutation lines approaching saturation of the transcriptome. This poster outlines our seed collection and distribution statistics.

Specifically: We currently distribute 30,000 tubes of seed a year. We sell more seeds in the UK than anywhere else, and together the UK and Germany make up nearly half of our total quantity for distribution.

The poster will give up-to-date statistics on the seed types that are currently popular and the changes in seed type requests over time. Most of our materials are knock-outs and other post genomic tools such as gene traps, enhancer traps, transactivation lines, and GFP fusions although we maintain large collections of RI populations, ecotypes and other non-transgenic materials.

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

534 Germplasm Customers and Donors

Craigon, Daugherty, Gill, Higgins, Humphreys, James, May, Okyere, Schildknecht,Sun
Nottingham Arabidopsis Stock Centre

NASC (est. 1991) services the European Arabidopsis community with seed and DNA resources. American users ordering from NASC are automatically and seamlessly transferred to the ABRC in Ohio for their orders to be fulfilled. Other nationalities choose their affiliation such that both stock centres service Asian, African and Australasian users depending on which stock centre the user visits first in each year.

This poster details our customer base. It outlines where our customers live, and the geographic distribution of large donors of stocks to the Stock Centre. Most of our donors are from the UK and US although we do have a large number of donations from a variety of other nationalities.

The advantages to the users of donating seeds to the stockcentre are:

Security: We preserve the seeds under the best possible conditions for longevity and germination frequency (*see: Fiona R. Hay, Andrew Mead, Kirstine Manger and Fiona J. Wilson. (2003) One-step analysis of seed storage data and the longevity of Arabidopsis thaliana seeds. J. Exp Bot. 54: 384, pp993-1011*)

Stability: Funded from 1991-2007 (and counting). Any seeds donated can be recovered if your local stocks get compromised or lost (or just lose their germination state) many years after you have donated them.

Publication: Many journals now require seeds to be donated to the stockcentres before publication. We will give you a NASC code (which is also an ABRC code) on receipt of the seeds.

Less work: We distribute on your behalf, and your name is associated with the donated stock forever.

Publicity: We will associate your stocks with documents from your lab, e.g. instructions on use, observations etc.

If you have seed to donate, please contact either of the stock centres or use our web pages. NASC can be contacted directly through **Arabidopsis@arabidopsis.info.**

535 NASC Data Services

Craigon, Daugherty, Gill, Higgins, Humphreys, James, May, Okyere, Schildknecht, & Sun
Nottingham Arabidopsis Stock Centre

The UK GARNet program was created to establish UK-based facilities for genomics research on Arabidopsis. All GARNet services and resources are publicly available and the aim is to create high quality (inter)national facilities of genomics resources for Arabidopsis and other plant research. Within GARNet, NASC has been tasked with multiple transcriptomics and bioinformatics outputs.

Our long standing genomics database (since 1996) is AGR. This contains sequence data from Arabidopsis linked to germplasm and other resources at NASC. Among other things, it is designed to allow easy searching for knockouts. AGR was built as a part of the UKCrop.net project and has been modified to include GARNet data. Information in AGR is intended to be oriented towards comparative analysis between Arabidopsis and other crop plants. It is closely integrated with the NASC Arabidopsis stock catalogue. NASC now also has a transcriptomics database (see associated poster) which is becoming integrated with our other databases to improve user choice and functionality.

Our user-oriented InsertWatch service (http://arabidopsis.org.uk/insertwatch) allows automated searching of new sequenced insert populations. Confidential matches are e-mailed back to the user as-and-when knockouts become available at the stock centres without requiring additional input from the customer.

New developments:

The current seed catalogue at NASC is undergoing renovation to improve the status as a data resource with emphasis on ease of use and interoperability with external databases via the European PLANet programme: Phenomics data will be made available to PLANet via BioMOBY

An XML file will be available for each seed stock and made available via web-services to external databases for general use.

Transcriptomics data generated in-house will also be made available to PLANet via BioMOBY and similar mechanisms. In addition, functional MAGE-ML will be generated for all our Affymetrix data. By the time of the Madison 2003 meeting, array data will have been donated to ArrayExpress at the EBI.

536 NASC arrays

Craigon, Daugherty, Gill, Higgins, Humphreys, James, May, Okyere, Schildknecht, Sun
Nottingham Arabidopsis Stock Centre

Established in 1991, the Nottingham Arabidopsis Stock Centre (NASC) provides various public services to the world Arabidopsis community (mainly seeds and data).

Since February 2002 we have been running a subsidised Affymetrix service facility whereby users from the international community send RNA samples to us to be hybridized onto Arabidopsis chips. Experiments are accepted from the community on a rolling programme through our web-pages. The proposals are peer-reviewed by an outside body for scientific merit and experimental design and then approved for processing.

We regularly advise users on the principle of biological standardization, good controls, careful ontologies and appropriate pooling. We encourage our service users to adhere to good MIAME standards and detailed recording and submission of appropriate supporting documentation into the database.

Our main drive is to make data from the chips publicly available as soon as possible in our own web database (AGR: NARRAYS) and to provide tools for analysis of the data. We now have public results from approximately 500 chip hybridizations available on-line and have been developing a local MAGE-ML solution in order to manipulate these data into ArrayExpress. Some data may have short (3 to 6 month) delays in release due to customer confidentiality.

Our self derived web-based tools are mainly associated with searching the data, and integrating it into our other databases. These include a spot-history tool to analyse the performance of single genes over all of the experiments held in our database. Because our users are in many countries, we emphasise the use of free tools.

All Arabidopsis data generated in our service is publically available and will additionally be exploited as part of our Exploiting Genomics initiative collaboration.

AffyWatch:

We distribute CDs which contain gene expression data produced from each experiment, along with full MIAME annotation. These are subject to a small cost recovery subscription.

537 Public Access to Microarray Data from the Arabidopsis Information Resource

Margarita Garcia-Hernandez, Nick Moseyko, Neil Miller, Dan Weems, Sue Rhee
Carnegie Institution of Washington , National Center for Genomic Research

The Arabidopsis Information Resource (TAIR) now provides access to microarray data. The first set of data comes from the Arabidopsis Functional Genomics Consortium (AFGC), which was obtained from the Stanford Microarray Database (SMD) and subjected to extensive curation and annotation at TAIR. Curation was applied at three levels: experiment, RNA samples, and expression results. At the experiment level, the individual hybridizations (516) were first grouped into experiment sets (123), then categorized and annotated using controlled vocabulary. The sample information was extracted from the RNA forms submitted by AFGC users (277 hybridizations) and from the channel's description available in SMD (516). Subsequently, the sample data was standardized and structured to allow searching by different parameters, including tissue, treatment, or germplasm. At the expression results level, the first round of curation included the sub-array, 'lowess' normalization of the data. We provide SMD's global normalization and our sub-array normalization data as well as the ANalysis Of VAriance (ANOVA) values for sector and plate-based spatial bias from both normalizations. The sub-array normalization reduced spatial bias significantly. In addition, we provide the arithmetic mean and related statistical measures of expression ratio values of each gene per replicate hybridization, experiment set, or across all AFGC arrays. Researchers can search for raw and normalized data using experiment description and experimenter information, download the raw and normalized results, download TAIR's all-by-all clustered data, and visualize the clusters using VxInsight. VxInsight software allows 3-D visualization from a bird's eye perspective on all the microarray data available in the TAIR database. The VxInsight viewer can be downloaded for free from the TAIR site along with accompanying microarray data. In addition, we provide continual updates to the genome mapping of the publicly available array designs (e.g. AFGC, Affy 8K, and Affy 23K). The genome mapping can be searched in bulk mode and the results are hyperlinked to our interactive expression viewer and SMD's spot history, and can also be downloaded as text files. Results from this work and future directions will be presented at the meeting.

538 Functional genomics resources for Arabidopsis systems biology approaches

Pierre Hilson, Lieve De Veylder, Tom Beeckman, Pierre Rouzé, Yves Van De Peer, Paul Van Hummelen, Martin Kuiper, Marc Zabeau, Dirk Inzé
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The complete sequence of the Arabidopsis nuclear genome has been available since December 2000. Yet, we have experimental proof of function for less than 10% of its annotated genes. We will describe tools being built on the basis of the genome sequence information and designed to accelerate the discovery of gene function. In the context of the CATMA consortium, we have in our hands a collection of over 24,000 Arabidopsis Gene Specific Tags (GSTs) originally designed for transcription profiling microarray analysis. Because reverse genetic approaches are extremely powerful and are required in large-scale gene function studies, the GSTs are being converted in a large array of constructs for the systematic silencing of each Arabidopsis gene via RNA interference. In parallel, in collaboration with other European laboratories, we have initiated the construction of two additional collections: open reading frames (ORFs) at the root of many functional proteomics studies and promoters for the investigation of transcription regulatory networks. These sequence repertoires (GST, ORF, promoter) are compatible with the Gateway recombinational cloning system and can be shuttled between vectors designed for specific functional assays using robust high throughput automated protocols. A comprehensive catalogue of such vectors is continually being improved. The sequence repertoires are tightly linked to the activity of multiple groups in the Department of Plant Systems Biology. They draw on the evolving gene and genome annotations, they are components of the bioinformatics toolbox and they feed the research in dedicated groups by promoting the functional analyses of large gene sets focusing on specific biological themes, including cell cycle, cell death and leaf and root development. We will illustrate with a few examples how this integration is currently taking place and why it is crucial to our understanding of entire biological systems.

Web links:

CATMA: www.catma.org; AGRIKOLA: www.agrikola.org; ORFEUS: www.orfeome.org
Department of Plant Systems Biology: www.psb.rug.ac.be
Gateway vectors for plant transformation: www.psb.rug.ac.be/gateway

539 The AGRIKOLA project - RNAi for Arabidopsis functional genomics

Pierre Hilson, Ian Small, Claire Lurin, Jim Beynon, Mansour Karimi, Javier Paz-Ares, Murray Grant, Thomas Altmann, Peter Waterhouse, Chris Helliwell, Ian Moore and collaborators

www.agrikola.com

Reverse genetics approaches based on targeted disruption of gene expression are powerful tools for determining gene function. In that perspective, post-transcriptional gene-silencing (PTGS) is udefull because it can be artificially trigerred by "RNA interference" (RNAi), the production of double strand RNAs from specifically designed transgene, the most efficient RNAs being the "hairpin" type (hpRNA). To create a gene encoding a hpRNA, we chose to use a short region of the target transcript that does not contain significant sequence similarity to other transcripts in the cell to avoid cross-silencing. First, we clone such specific gene-sequence tags (GSTs, 150-600bp long) generated in the framework of the CATMA project (www.catma.org) for as many of the 25000+ Arabidopsis genes as possible. Then, GSTs are subcloned via high-throughput recombinational cloning techniques on both sides of a hpRNA intron spacer and in inverted orientation to construct a gene expressing the corresponding double strand RNAs. To facilitate the analysis of genes whose mutation is lethal or leads to a very severe phenotype, we will next create a second-generation of inducible hpRNA vectors. The AGRIKOLA plasmid collections will be made available to the scientific community via the Nottingham Arabidopsis Stock Centre.

Although sufficient is known to assess that the hpRNA approach is generally effective, no systematic attempts to carry out gene-silencing have yet been undertaken in plants. We will study the effects of RNAi in Arabidopsis plants on a large scale by transforming 4000 constitutive hpRNA clones covering chosen genes and 1000 inducible hpRNA clones and examining the resulting phenotypes at a basic visual level. We will gather useful information on the efficacy of the approach and the types of recovered phenotypes, including the proportion of clones which generate severe or lethal phenotypes. Furthermore, we will analyse in detail 150-200 selected transformed lines silenced for genes of particular interest and compare silencing triggered by constitutive and inducible hpRNA constructs. These additional experiments will help identify the function of important Arabidopsis genes for which mutants are not currently available.

540 TAIRway to Heaven - New Tools and Data at TAIR

The Arabidopsis Information Resource (See Footnote for Authors)

Carnegie Institution and National Center for Genome Resources

The Arabidopsis Information Resource (TAIR) (www.arabidopsis.org) presents researchers with a comprehensive data resource, integrating information on genes, proteins, maps, clones, seed and DNA stocks, biochemical pathways, community members and literature. We have recently added T-DNA and transposon insertions to the TAIR Sequence Viewer, which also displays genes, clones, ESTs, full-length cDNAs, genetic markers and polymorphisms on a searchable and zoomable map of the Arabidopsis genome. We have also added a simple chromosome map tool that can be used to draw custom maps of the Arabidopsis genome using a list of locus names. In the past few months we have introduced 5 new search tools: a polymorphism and allele search tool for finding SNPs, T-DNAs, deletions and alleles; a germplasm search tool for locating T-DNA, transposon insertion and TILLing lines, transgenic lines and other mutant and mapping strains; an ecotype search tool; a protein search tool for finding proteins based on physical properties, domains, gene names or map information; and a microarray search tool. Most AFGC (Arabidopsis Functional Genomic Consortium) microarray data have now been incorporated into TAIR and are currently searchable and downloadable by experiment set. Searching by gene, expression level, and keywords describing the RNA source will be added soon. One of TAIR's ongoing efforts is the literature-based annotation of Arabidopsis genes using a structured vocabulary for biological process, cellular component, molecular function, anatomical part and developmental stage that is being developed by TAIR in collaboration with other database groups, including the Gene Ontology (GO) Consortium and the Plant Ontology Consortium. Another ongoing effort is the curation of biochemical pathway data available in TAIR's Aracyc tool, which includes information on enzymes, compounds, reactions and genes. In collaboration with the Multinational Arabidopsis Steering Committee (MASC), TAIR also provides a gateway to the latest information and news about the functional genomics efforts around the world. In addition to maintaining a list of funded projects, TAIR provides a searchable and downloadable list of genes that are under investigation. Currently, 24356 unique loci are targeted for study by 36 projects that have provided their gene list to TAIR.

Huala E, Berardini TZ, Chen G, Doyle A, Garcia-Hernandez M, Lander G, Mahini B, Miller N, Montoya M, Moseyko N, Mueller LA, Mundodi S, Nunn R, Reiser L, Tacklind J, Weems DC, Xu I, Yoo D, Yoon J, Zhang P, Rhee SY.

541 High Throughput Production of *Arabidopsis thaliana* Proteins for Structural Genomics

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An efficient and flexible pipeline system has been developed that allows high throughput purification of *Arabidopsis thaliana* target proteins expressed in *E. coli*. The key steps of the protein pipeline include (1) initial purification of S-peptide-His6-MBP-tagged fusion proteins from cell lysates; (2) TEV protease cleavage; (3) removal of the liberated S-peptide-His6-MBP tag from the target protein; and (4) target protein evaluation and concentration. In order to take advantage of the possibilities for throughput scaling offered by automation, we have developed protocols for a 2D mode HPLC system equipped with a sample pump and 6 valves to perform serial 2 step column chromatography. Protocols using both batch and gradient elution have been implemented. Sets of 6 different protein samples are loaded in parallel onto Ni-IDA columns with a programmable sample pump. Following elution from the first Ni-IDA column, the His-tagged fusion proteins are directly transferred to desalting column. By using UV/vis detection, the desalted proteins are automatically collected on fraction collector. The purified fusion proteins are treated with TEV protease and the target proteins are separated from His-tagged proteins by using subtractive Ni-IDA columns chromatography. Optimized protocols for the purification of 15N-, 13C and 15N-, and SeMet-labeled proteins with isotopic enrichment of up to 97% and with purity suitable for structural determination by X-ray and NMR are also available. An on-line, interactive laboratory information management system [the Lamp module of the Sesame software package, (1)] is utilized for data capture and analysis, allowing efficient assessment of quality control during purification processes. The Sesame package also provides storage and retrieval of biochemical data on purified proteins including SDS-polyacrylamide gels, UV-visible spectra, and results of MALDI-MS and ESI-MS analyses. This project is supported by NIH grant P50 GM64598.

1. Z. Zolnai et al., J. Structural and Functional Genomics 2, 181-193 (2002).

542 AGRIS, Arabidopsis Gene Regulatory Information Server, a first step in establishing regulatory networks in plants

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Gene regulatory information is encoded in the promoter regions formed by *cis*-regulatory elements that bind specific transcription factors (TFs). Hence, establishing the architecture of plant promoters is fundamental to understanding gene expression. AGRIS is an information resource of *Arabidopsis* promoter sequences, TFs, and their target genes. AGRIS currently contains two databases, AtTFDB (*A. thaliana* TF database) and At*cis*DB (*A. thaliana cis*-regulatory database). AtTFDB contains approximately 1,500 transcription factors identified directly through BLAST and motif searches and arranged into 33 primary families. The user can browse through the families or perform a specific search using the Locus ID, or part of the TF description. Results will display the Locus ID, gene name if known, a description, and links to other Arabidopsis resources - TIGR, MIPS, SALK, and TAIR. The database also provides an opportunity to download the complete nucleotide or protein set of Arabidopsis TFs, the entire family alignment, and the family's Hidden Markov Model. At*cis*DB consists of the 5' regulatory sequences of all 27,975 annotated genes with a description of putative *cis*-regulatory elements. Users can search At*cis*DB using the Gene Name, Locus ID, or GenBank Accession Number. For each result, the user can view the promoter sequence in both directions, with a color-coded graphical display of the type of binding site predicted. Currently, we are linking specific TFs with their documented corresponding promoter sequences in an attempt to begin describing regulatory networks. AGRIS, is one of the first attempts to provide the necessary software tools to incorporate *Arabidopsis* TFs and their putative binding sites on all genes to initiate the identification of transcriptional regulatory networks. AGRIS can be accessed from http://arabidopsis.med.ohio-state.edu or though TAIR.

543 AraCyc, a biochemical pathway database for Arabidopsis thaliana

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1 TAIR, Carnegie Institution of Washington, 2 NCGR

AraCyc is a database being developed at the Arabidopsis Information Resource (TAIR) for the annotation of biochemical pathways in Arabidopsis thaliana. It features over 180 pathways that include information on reactions, compounds, enzymes, and genes. The reactions are annotated with conventional EC numbers, and virtually all compounds are associated with chemical structures and extensive synonyms. Physical properties (molecular weight and subcellular location) and catalytic properties are annotated to the enzymes. The enzyme-coding genes are linked to TAIR's gene detail pages. The pathways are generated dynamically from the underlying database and can be viewed graphically. A ?zoom in? feature reveals more details about the reactions down to the chemical structures of all compounds and co-factors. In addition, each reaction in a pathway is displayed with the corresponding enzymes and genes. An overview diagram gives a bird?s-eye view of all pathways in the database, and an expression-overlay function allows gene expression data to be overlaid on this diagram. The database is powered by the Pathway Tools software developed by Peter Karp?s group at SRI International. The latest release of Pathway Tools software features enhanced query for pathways, compounds, reactions, enzymes, and genes, and enhanced expression-overlay function to display results from time course experiments using the new animation feature. Users can also define their own color scales used by the expression-overlay function. Since the initial build of AraCyc, more than twenty-five pathways have been added from the literature. New pathway classes (plant hormone and secondary metabolism) were created with the addition of new pathways. New pathways were also added to the existing pathway classes such as energy metabolism (photorespiration), metabolism of storage carbohydrates (starch and fructan), lipid metabolism (lipid desaturation pathways, and biosynthesis of glycosylglycerides, wax and cutin), among others. In addition, a number of pathways have been updated and errors in some of them were corrected from user's feedback. AraCyc is available under the tools section of TAIR (http://www.arabidopsis.org/tools/aracyc). Current activities and future directions will be presented at the meeting.

544 TAIR 2 GO: Controlled Vocabularies and Functional Annotation at TAIR

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One of The Arabidopsis Information Resource's (TAIR's) current goals is to associate Arabidopsis genes with structured vocabulary terms developed by the Gene Ontology (GO) Consortium. We plan to annotate the entire Arabidopsis genome to GO vocabularies that describe the molecular function, biological process, and subcellular component of a gene product. As members of the GO consortium since 2000, TAIR has made significant contributions to developing and modifying the controlled vocabularies to accommodate annotations of plant gene products. In collaboration with other plant databases we have also developed vocabularies to describe plant anatomy and developmental stages and are using them to annotate published expression patterns of Arabidopsis genes. We plan to extend these vocabularies for use in other plant species under the auspices of the Plant Ontology Consortium. TAIR?s initial GO annotations used computational methods that provided a general, low resolution overview of the transcriptome. Our current emphasis is on using the experimental evidence in the existing literature to assign more granular GO terms to the approximately 10% of Arabidopsis genes that have been published in the literature (PubSearch, TAIR's literature curation tool). We have been collaborating closely with The Institute for Genome Research (TIGR) in the GO annotation effort and display our collective work on TAIR's gene and locus detail pages. Genes annotated with GO terms and expression patterns can now be searched using keywords. The GO annotation bulk download interface at http://www.arabidopsis.org/tools/bulk/go/index.htmlallows researchers to obtain GO annotations for any gene or set of genes using locus names. In addition,TAIR?s new keyword browser allows users to navigate through the ontology structures and explore term relationships and view definitions. The research community is strongly encouraged to review the annotations of their genes of interest and provide feedback to TAIR.

545 GARNet, The Genomic Arabidopsis Resource Network

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GARNet is part of the BBSRC Investigating Gene Function (IGF) initiative and provides (inter)national facilities and resources for Arabidopsis functional genomics research. The spearpoints of the GARNet project are: transcriptome analysis, bioinformatics, metabolite profiling, proteome analysis and forward and reverse genetics. The GARNet service providers undertake the development and maintenance of the different resources and facilities, providing the Arabidopsis community with access to high quality services. A price list for the current GARNet services will be display on the poster. Once a year an open meeting is organised for the research community. This year, the fourth annual GARNet meeting will take place from September 3 to 6 at the University of York, U.K. It will be combined with the 2nd Plant-GEMs meeting. Plant-GEMs, (Plant Genomic European Meetings) is a European meeting series on functional genomics and genome research in model plants and crops. It is organised by GARNet, and the Netherlands, German and French functional genomics programmes. Plant-GEMs aims not only to spread awareness and use of functional genomics technologies, but also to establish more links and collaborations between the different national programmes, and avoid unnecessary duplications of effort. We will have sessions on the following subjects: Highlights in Genomics, Genetic Networks underlying biological processes, Comparative genomics and evolution, Plants and improved nutritional quality, Genomics assisted Breeding, Genomics of development, Function of unknown genes, Plant Genomics and Abiotic Environment Plant Genomics and Biotic Interactions, Bioinformatics, Brassica genomics- from model to crop, Cereal functional genomics, Metabolomics, Transcriptomics, Proteomics, Genomics in signal transduction, Application-oriented genomics in non-model plant species. We think this will be a highly exciting meeting, and hope you can attend and contribute to it. Information on GARNet Services and the 2003 combined 2nd Plant-GEMs / 4th GARNet meeting and Plant-GEMs is available at http://garnet.arabidopsis.org.uk and http://plant-gems.org

546 A Web-based Functional Classification SuperViewer for Arabidopsis Genomics

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Functional classification is commonly applied to data generated by microarray gene expression profiling experiments. Indeed, one of the most often used bioinformatics tools after BLAST and clustering is the functional classification pie chart. However, presentation of the absolute numbers of genes in a given cluster falling into each functional classification category may be misleading or mask difference under a given treatment. Another way of examining this sort of information is to normalize to the number of genes in each class present on the chip. In this way, differences are more easily perceived. We present here a web-based tool, the Functional Classification SuperViewer, which performs this normalization, bootstraps the dataset to provide a confidence estimate for the accuracy of the output, generates a dynamic graph summarizing the output for easy incorporation into reports, and provides links to TAIR and other databases for individual IDs entered. Furthermore, expression values may also be submitted along with the IDs, and the values will be displayed with a colour-scale background, along with a functional classification bar-code.

547 Genomics-Related Stocks at ABRC

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The Arabidopsis Biological Resource Center (ABRC) maintains many stocks relevant to genome exploration and numbers of stocks are being received from NSF 2010 Project grant recipients. Among the stocks already being received are: a) SALK sequence indexed T-DNA lines; b) chromatin-related mutants; c) large numbers of embryo-defective mutants; d) related species, natural accessions; e) full length cDNA clones in the pUNI vector; f) BAC collections from species related to A. thaliana. A number of other donations are expected.

Two of the major genomic resources will be highlighted. First, essentially all of the 140,000-member T-DNA collection from the Ecker/Salk project has been received. These identify at least one insertion in 20,000+ genes of Arabidopsis. Further, multiple insertions have been found for many of these genes, since approximately 60,000 of the SALK lines have been annotated as having insertions in genes. The second major resource is the Full-length Open Reading Frame clones being received from the SSP Consortium (the R. Davis /J. Ecker/A. Theologis laboratories). 8,000+ of these clones have been received. The Cre-Lox based recombination system of these clones can be used to easily insert the basic cDNA clones of our collection into destination vectors having many different end uses.

In addition, we have available T-DNA lines, associated DNA pools, the BAC genomic clones utilized for the sequencing projects and the EST collection representing ca. 10,000 Arabidopsis genes. The T-DNAs employed to generate these lines include enhancer trap, activation tagging and over-expression constructions, as well as simple insertions.

ABRC is supported by the National Science Foundation.

548 The Arabidopsis Biological Resource Center - 2003 Stock Acquisition and Distribution Update

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The Arabidopsis Biological Resource Center (ABRC) cooperates with the Nottingham Arabidopsis Stock Centre (NASC) to collect, preserve and distribute seed and DNA stocks of Arabidopsis. ABRC stock can be ordered through the TAIR database Web site maintained by the Carnegie Institution of Washington (http://arabidopsis.org), with informatics support from the National Center for Genomic Resources (NCGR).

Diverse new seed stocks have been added to our collections in the past year, including: A) flank-tagged T-DNA and transposon insertion lines, B) mutant and transgenic lines, C) natural accessions, and D) representatives of additional new related species. The T-DNA insertion of the SALK collection presently includes lines having insertions in 20,000+ different Arabidopsis genes.

New DNA stocks added to the collection include: A) open Reading Frame (ORF) clones from the SSP Consortium, B) clone accessions, C) RNAi vectors and D) libraries, including BAC libraries from four related species.

During the past year, ABRC distributed 60,000+ seed and 20,000 DNA stocks to researchers.

ABRC is supported by the National Science Foundation.

549 Sesame project management system for structural genomics

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Sesame is a web-based software package (www.sesame.wisc.edu) designed to (1) organize data relevant to the overall management of a complex scientific project, (2) record information on the methodology employed in individual steps, (3) launch computer-controlled processes, (4) make decisions about subsequent steps on the basis of information returned, (5) harvest and archive intermediate and final results, (6) provide detailed web-accessible views of the project to those with need to know, and (7) organize data for publication, deposition in databases, or posting on web sites. The Sesame software package, which follows the client/server paradigm, consists of a *framework*, which supports secure interactions among the three tiers of the system (the *client*, *server*, and *database* tiers), and *application modules* that carry out specific tasks. For security, access to stored data is controlled by access privileges set by the owners of the data. We will present several Sesame modules that have been developed to support steps involved in structural and functional proteomics. This project is supported in part by NIH grant P50 GM64598.

550 Large-Scale Discovery of Induced Point Mutations by the Arabidopsis TILLING Project

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TILLING (Targeting Induced Local Lesions in Genomes) is a general reverse-genetic strategy that provides an allelic series of induced point mutations in genes of interest. High-throughput TILLING allows the rapid and low-cost discovery of induced point mutations in populations of chemically mutagenized individuals. As chemical mutagenesis is widely applicable and mutation detection for TILLING is dependent only on sufficient yield of PCR products, TILLING can be applied to most organisms. We have developed TILLING as a service to the Arabidopsis community known as the Arabidopsis TILLING Project (ATP, http://tilling.fhcrc.org:9366/). Our goal is to rapidly deliver allelic series of ethylmethanesulfonate-induced mutations in target 1-kb loci requested by the international research community. At the time of this abstract, ATP has delivered more than 2200 mutations in over 220 genes ordered by Arabidopsis researchers. Analysis of these data confirms that the mutations available in our reference population are efficiently detected by high-throughput TILLING and that the full spectrum of point mutations, including the more severe deleterious missense and knockout lesions, are recovered. One additional goal of ATP is to disseminate TILLING technology to the broader biology community for application to other organisms. To facilitate this, we regularly hold 2-day workshops at our TILLING lab in Seattle, Washington.

551 High Throughput Cloning of *Arabidopsis* cDNAs at the Center for Eukaryotic Structural Genomics (CESG).

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The Center for Eukaryotic Structural Genomics (CESG) was founded as a collaborative effort to develop critical technologies for determining three-dimensional structures of proteins rapidly and economically. CESG's initial focus is on the genome of the model plant *Arabidopsis thaliana*. This poster will focus on the high throughput methods that we have developed for cloning Arabidopsis open reading frames (ORFs) into *E. coli* expression vectors. ORFs are chosen on the likelihood that they will open up important regions of protein conformation and fold space or that they will elucidate novel fold-function relationships. CESG will also consider proteins of structural or functional interest suggested by the plant science community. Gene chips produced by maskless array DNA synthesis are used to determine the presence of targeted ORFs in cDNA pools generated by reverse transcription of RNA isolated from an *Arabidopsis* callus cell line. CESG's standard cloning protocol utilizes Invitrogens's 'Gateway' plasmid construction system to clone the PCR amplified ORFs into various expression vectors containing different promoter systems and protein tags. Technology and products developed by CESG, including clones, expression vectors, and excess protein, are available to the scientific community through establishment of collaborative research agreements. This project is supported as a Pilot Project in the NIH Protein Structure Initiative under Grant P50 GM64598 from the National Institute of General Medical Sciences.

552 High throughput generation of full-length cDNA sequences for Arabidopsis ge

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We are interested in elucidating the structure and function of Arabidopsis "hypothetical genes" -those that have no known homologs outside Arabidopsis and are based only upon computer prediction, requiring two concordant predictions to be included in the current TIGR genome annotation. Recently, we have also used comparative genomic analysis of ~450,000 whole genome shotgun reads from Brassica to infer the existence of additional genes that have neither computational nor database support. Our analysis indicates the presence of more than 10,000 regions of sequence conservation in the parts of the Arabidopsis genome not presently annotated, suggesting the existence of many novel genes that also lack robust in silico predictions. At TIGR, we have developed a high throughput method for the cloning and analysis of these "twilight zone" genes. To test their expression, we used cDNA populations from a number of plant tissues and treatments. Of the 893 hypothetical genes tested, 596 are expressed in our cDNA mixture. Of the first set of 192 genes predicted by comparative genomics, 57 are expressed. The full-length cDNA sequences of these predicted genes were obtained by sequencing and assembling their 5' and 3' RACE products. So far, we have obtained a total 506 full-length or partial cDNA sequences of Arabidopsis hypothetical genes and 50 genes predicted by Brassica sequence conservation. The cDNA sequences from 212 hypothetical genes display differences from their predicted gene structures. In total, 83 hypothetical genes have alternatively spliced transcripts and 103 genes display more than one polyadenylation site. Thus many of both the hypothetical and Brassica-predicted genes are expressed. Functional analysis of these genes is in progress.

553 New Gene Symbol Registration Functionality at TAIR

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To avoid naming conflicts, it is important that there be a central place for registering gene name symbols specific to an organism. An aspiring symbol should be checked against a large database of symbols that are used in the literature or that are already registered, and then assessed by experts for appropriateness and ambiguity. In the past, Prof. David Meinke has maintained such a registering process for Arabidopsis genes with mutant phenotypes. With the help of David Meinke, TAIR is now integrating this function into the TAIR database and extending the registration to Arabidopsis genes without known mutant phenotypes. On this poster, we describe the basic functionality of the registration process, outline how it is implemented and summarize some of the gene symbol data presently stored in the TAIR database. Please visit TAIR at http://www.arabidopsis.org to learn more about registering gene symbols and Arabidopsis gene nomenclature.