1 Hypothesis building by plant metabolomic analysis

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Now that the complete Arabidopsis genome sequence is available, functional genomics aims to assign functions to the encoded plant genes by accurately determining the resulting phenotypes. One way to do so is by assigning 'molecular phenotypes', i.e. quantifying the regulation of levels of mRNA, proteins, or metabolites with respect to genetic or environmental changes.

Metabolites can be regarded as end products of genes precisely defining the net result of all cellular regulation processes. Therefore, comprehensive analysis of the metabolome might enable an in-depth understanding of the biochemical response upon genetic perturbations. Several thousands of metabolites may appear simultaneously in Ararbidopsis plants. Methods for sample preparation are compared, and examples are given for metabolomic profiles from different plant species. By use of mass spectrometry, over 1,000 metabolites are currently detectable from a single Arabidopsis leaf, with 50 samples analysed per day. Whereas GC/TOF MS proves to be highly advantageous concerning speed, resolution, and reliability of data acquisition, identification of unknown compounds is easier to achieve by LC/MS/MS. Different concepts for finding, identifying, quantifying, and normalizing metabolites in raw data files are presented, with special emphasis on routine processes.

Therefore, the bottleneck of metabolomic analysis is not data acquisition, but sample preparation and data analysis. The application of bioinformatic tools is exemplified by the analysis of metabolomic data sets gained from Arabidopsis mutants, and crosses between wild type accessions. Statistics, web based databanks, supervised and non-supervised learning algorithms, correlation analysis, and network generation is used to generate novel hypotheses on plant metabolism, including efforts to link metabolomic data back to genomic information.

Potential applications of metabolomic analysis in plant biotechnology might include metabolic engineering or assessing the 'substantial equivalence' of GMO foods. For better understanding of regulatory networks in general, as well as interactions with protein and gene regulation, metabolomic analysis will be a further key stone to in silico descriptions of Arabidopsis plants, complementary to transcriptomic and proteomic phenotyping.

2 A Fast Neutron Deletion Mutagenesis-based Reverse Genetics System for Plants

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A new reverse genetics method has been developed to identify and isolate deletion mutants for targeted plant genes. Deletion mutant libraries are generated using fast neutron bombardment. DNA samples extracted from the deletion libraries are used to screen for deletion mutants by polymerase chain reaction (PCR) using specific primers flanking the targeted genes. By adjusting PCR conditions to preferentially amplify the deletion alleles, deletion mutants were identified in pools of DNA samples with each pool containing DNA from 2,592 mutant lines. Deletion mutants were obtained for greater than 80% of targeted loci from an Arabidopsis population of 51,840 M2 families. A large number of deletion mutants have been identified and multiple deletion alleles are often recovered for targeted loci. By isolating deletion mutants for genes with a wide range of sizes, we demonstrated that the method is very useful for targeting small genes. In addition, we have showed that it is possible to find deletion mutants mutating two or three tandem homologous genes. Data on molecular and phenotypic analysis of these mutants will be presented. We also showed that it is possible to apply this method to plant species other than Arabidopsis by isolating a deletion mutant for a rice gene using a similar approach. Since fast neutron mutagenesis is highly efficient, it is practical to develop deletion mutant populations with more complete coverage of the genome than methods based on insertional mutagenesis. Because fast neutron mutagenesis is applicable to all plant genetic systems, this method has the potential to enable reverse genetics for a wide range of plant species.

3 Construct Design for Efficient, Effective and High Throughput Gene Silencing in Plants

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Post-transcriptional silencing of plant genes using antisense or co-suppression constructs usually results in only a modest proportion of silenced individuals. Recent work has demonstrated the potential for constructs encoding self-complementary "hairpin" RNA (hpRNA) to efficiently silence genes (Smith *et al., Nature* 2000, 407, 319-320). In this study we examine design rules for efficient gene silencing both in terms of the proportion of independent transgenic plants showing silencing and the degree of silencing. Using hpRNA constructs containing sense/antisense arms ranging from 98 to 853 nts gave efficient silencing in a wide range of plant species and inclusion of an intron in these constructs had a consistently enhancing effect. Intron-containing constructs (ihpRNA) generally gave 90-100% of independent transgenic plants showing silencing. The degree of silencing with these constructs was much greater than that obtained using either co-suppression or antisense constructs. We have made a generic vector, pHANNIBAL, that allows a simple, single PCR product from a gene of interest to be easily converted into a highly effective ihpRNA silencing construct. We have also created a high-throughput vector, pHELLSGATE, that should facilitate the cloning of gene libraries or large numbers of defined genes, such as those in EST collections, using an *in vitro* recombinase system. This system may facilitate the large-scale determination and discovery of plant gene functions in the same way as RNAi is being used to examine gene function in *C. elegans* (Fraser *et al., Nature* 2000, 408, 331-336).

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4 Sulfolipid Biosynthesis: The joining of sulfur, sugar, and lipid metabolism to produce a unique anionic sulfolipid in thylakoid membranes of *Arabidopsis thaliana*

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Sulfoquinovosyl diaclyglycerol (SQDG) is commonly found in the photosynthetic membranes of higher plants and bacteria, is one of the most abundant sulfur-organic compounds on earth, is an excellent detergent, and has prospects as anti-viral and anti-cancer agents. The sulfolipid biosynthetic pathway in *Arabidopsis thaliana* has been elucidated and is composed of two unique enzymes: UDP-SQ synthase (SQD1) and UDP-SQ:DAG sulfoquinovosyltransferase (SQD2). The SQD1 gene has been cloned, expressed, and the protein has been crystalized. Enzyme assays have identified the source of the sulfur donated to create the sulfonic acid (Sanda et al., 2001 *J Biol Chem* **276**: 3941-3946; Mulichak et al., 1999 *PNAS* **96**: 13097-13102). SQD1 synthesizes UDP-sulfoquinovose in the chloroplast utilizing UDP-glucose and sulfite, the product of APS reductase from the sulfate reducing pathway. Enzymatic parameters have been established and site directed mutagenesis has exemplified the reaction mechanism to be similar to members of the short chain dehydrogenase/reductase family. SQD2 has recently been cloned from *Arabidopsis* and consists of a membrane bound glycosyl transferase which transfers sulfoquinovosyl onto diacylglycerol. T-DNA insertional inactivation of SQD2 in *Arabidopsis* results in plants with no detectable SQDG. Co-expression of SQD1 and SQD2 in *E. coli* leads to reconstitution of SQDG biosynthesis in this bacterium. These results establish a complete pathway for the production of sulfolipid in chloroplasts.

5 Drastically altered xyloglucan structure in the arabidopsis cell wall mutant *mur3*

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Xyloglucans are the principal cross-linking glycans in the primary cell walls of most flowering plants. The physical interaction between xyloglucans and cellulose microfibrils is a major biophysical determinant of cell wall architecture and its alteration during cell growth. With few exceptions, xyloglucans contain fucosylated trisaccharides attached at regular positions along the glucan backbone, and several hypotheses have been forwarded to explain their function, such as efficient binding of xyloglucan to cellulose or oligosaccharide-mediated regulation of hormone-induced growth. Xyloglucan from the arabidopsis cell wall mutant *mur3* lacks the fucosyl-galactosyl side chain normally attached to a xylose residue leading to a greatly simplified polysaccharide structure. Positional cloning of the *MUR3* gene revealed that it encodes a type II membrane protein similar to Golgi-localized glycosyltransferases. MUR3 protein expressed in a mammalian cell line was able to galactosylate xyloglucan from *mur3* plants indicating that it functions as a galactosyltransferase specific for the third xylose residue within the XXXG unit of xyloglucans. Despite the synthesis of a severely altered xyloglucan, the morphology of the plants and cell structure appear indistinguishable from wild-type. Furthermore, the strength of the primary cell wall in elongating inflorescence stems was the same in wild type and mutant plants. These data cast serious doubts on the proposed role of the xyloglucan trisaccharide side chain for cell wall assembly and the generation of signal molecules. They also illustrate that plants are surprisingly tolerant to drastic changes in major cell wall polysaccharides with interesting implications for plant biotechnology. Supported by NSF grant MCB-9728779 (to W.-D. R.) and a grant from the USDA-NRICGP (to N.C.).

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6 *S*-Adenosylmethionine is a Likely Effector for the Feedback Regulation of the CGS mRNA Stability of Cystathionine γ-Synthase

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Through a course of studies with Arabidopsis *mto1* mutants that overaccumulate soluble methionine (Met), we have shown that expression of cystathionine γ-synthase (CGS), the key enzyme in methionine (Met) biosynthesis, is regulated at the level of mRNA stability in response to Met or its metabolites in Arabidopsis, and that an amino acid sequence encoded by the first exon of the *CGS* gene has an important role for this regulation [Chiba et al. (1999) Science 286: 1371-1374]. In transfection experiments with Arabidopsis callus, exogenously applied Met showed a stronger effect on the regulation than exogenously applied *S*-adenosylmethionine (SAM), the first product of Met metabolism. However, this might be due to a difference of efficiency of incorporation into cells between Met and SAM. We developed an *in vitro* assay system with wheat germ extracts to explore the effector of the regulation of CGS mRNA stability. In contrast to the transfection experiments, SAM showed a much stronger effect on the regulation than Met in experiments using the *in vitro* assay system. This result suggests that a metabolite of Met is the effector rather than Met itself and that, in the transfection experiments, exogenously applied Met worked after being converted to its metabolites. Effect of *S*-adenosylhomocysteine (SAH), a next product of SAM in the Met metabolism, was also tested using the *in vitro* assay system. SAH showed no effect on the regulation. Therefore, so far, SAM is the best candidate for the effector of the regulation of the CGS mRNA stability.

7 Integration of sugar-sensing and plant hormone signalling pathways in the regulation of starch biosynthesis in Arabidopsis

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Plants both produce and utilise carbohydrates and have developed mechanisms to regulate their sugar status and co-ordinate carbohydrate partitioning. High sugar levels result in a feedback inhibition of photosynthesis and an induction of storage processes. We used a genetic approach to isolate components of the signalling pathway regulating the induction of starch biosynthesis. The regulatory sequences of the sugar inducible ADP-glucose pyrophosphorylase subunit ApL3 were fused to a negative selection marker. Of the four *impaired sucrose induction* (*isi*) mutants described here, two (*isi1* and *isi2*) were specific to this screen. The other two mutants (*isi3* and *isi4*) showed additional phenotypes associated with sugar-sensing screens that select for seedling establishment on high sugar media. The *isi3* and *isi4* mutants were found to be involved in the abscisic acid signalling pathway. *Isi3* is allelic to *abscisic acid insensitive4* (*abi4*), a gene encoding an Apetala2 type transcription factor. *Isi4* is allelic to *glucose insensitive1* (*gin1*) and these mutants were identical to the ABA biosynthetic mutant *aba2*, which was found to encode a short-chain dehydrogenase/reductase. Expression analysis showed that ABA is unable to induce *ApL3* gene expression by itself, but greatly enhances *ApL3* induction by sugar. Our data suggest a major role for ABA in relation to sugar signalling pathways in that it enhances the ability of tissues to respond to subsequent sugar signals.

8 Starch in excess: What affects the *Arabidopsis thaliana* mutant *sex1*?

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Starch is the major storage carbohydrate in higher plants and is used as a raw material for processing food, pharmaceutics, cosmetics, paper and plastics. While plant starch biosynthetic pathways are well characterised, our understanding of starch degradation is rather incomplete. Mutants selected for a high starch content after prolonged dark periods provide excellent tools to study the diurnal turnover of starch. One important mutant of this starch excess type is the *Arabidopsis thaliana sex1* mutant. The EMS mutagenised allele *sex1-1* (= TC265) accumulates three to five times as much leaf starch compared to the corresponding wild-type (Caspar et al., 1991). This mutant is impaired in the mobilisation of assimilatory starch at night and was supposed to be defect in the plastidic glucose translocator pGlcT (Trethewey and ap Rees, 1994a, b). Recently, the putative chloroplast hexose transporter was isolated and characterised (Weber et al., 2000). Surprisingly, the *sex1-1* mutant phenotype could not be complemented by introducing a wild-type copy of the spinach pGlcT gene. By mapping the *sex1-1* locus and cloning the corresponding gene, *sex1-1* turned out to be defective in a gene that encodes a protein with significant homology to the reversibly starch-granule binding protein R1 from potato (Lorberth et al., 1998). This protein influences the degree of starch phosphorylation in leaves and tubers of potato (Lorberth et al., 1998). A downregulation of R1 in potato led to a strong reduction of the amount of phosphate of amylopectin (Lorberth et al., 1998). However, the precise reaction catalysed by R1 is still under investigation.

Caspar T., Lin T.-P., Kakefuda G., Benbow L., Preiss J., Somerville C. (1991); Plant Physiol. 95: 1181-1188. Lorberth R., Ritte G., Willmitzer L. and Kossmann J. (1998); Nature Biotech. 16: 473-477. Trethewey R. N., ap Rees T. (1994a); Biochem. J. 301: 449-454. Trethewey R. N., ap Rees T. (1994b); Planta 195: 168-174. Weber A., Servaites J. C., Geiger D. R., Kofler H., Hille D., Gröner F., Hebbeker U. and Flügge U.-I. (2000); Plant Cell 12: 787-801.

9 Developmental Regulation of Flavonoid Biosynthesis and Compartmentation in Arabidopsis Seed Coat

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Seed coat flavonoids, particularly condensed tannins (proanthocyanidins) and flavonols, have an important impact on seed quality, considering both agronomic and nutritional aspects. Engineering flavonoid metabolism specifically in seed coats is therefore attractive, but a preliminary requirement for this is a thorough understanding of how the biosynthesis and compartmentation of flavonoids are genetically controlled. Our laboratory has been taking a mutant approach based on a visual screening of seed color to identify genes involved in the flavonoid biosynthesis pathway. Until now, the Versailles T-DNA transformant collection has yielded 23 seed pigmentation mutants representing 14 different loci. Recently, 8 additional mutants were isolated and are being characterized genetically. In the framework of a reverse genetics approach, we will take benefit from the Versailles FST (Flanking Sequence Tag) collection to look for mutants in genes of the flavonoid pathway which sequence is known. T-DNA-tagged alleles of *TT8* and *TT2* enabled the cloning of the genes that encode transcription factors of the bHLH type and of the MYB type, respectively. These genes were shown to regulate the flavonoid late biosynthetic genes *DFR*, *BAN* and *TT12* in siliques. The *TT12* gene was also cloned and encodes a putative flavonoid transporter that might direct flavonoids to the vacuole of endothelial cells. The molecular characterization of 2 other genes, exhibiting a significant protein sequence homology with a transcription factor and a UDP-glucose:sterol glucosyltransferase, respectively, is in progress. Yeast two-hybrid assays are underway to assess whether the TT8 and TT2 regulatory proteins interact with each other, and with the TTG1 protein. In parallel, the functional analysis of promoters from some flavonoid biosynthetic genes has been undertaken, using both the GUS reporter gene for expression in planta and the yeast one-hybrid system to look for factors interacting with these promoters.

10 Biochemical evidence for a role of the COP1 interacting protein 8 (CIP8) in ubiquitination of the HY5 transcription factor

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The Arabidopsis transcription factor HY5 is a positive regulator of seedling photomorphogenesis. HY5 protein strongly accumulates in response to light stimulus. Targeted degradation via the proteasome pathway has been shown to be the likely mechanism that keeps HY5 levels low in the dark. HY5 physically interacts with COP1, a negative regulator of photomorphogenesis. Degradation of HY5 depends on the presence of COP1, and consistently COP1 is nuclear localized only in darkness. Based on these observations, it has been suggested that COP1 targets HY5 for degradation via the proteasome pathway. In particular, the presence of multiple protein interaction domains in COP1 qualifies it as a possible component of an E3 ubiquitin ligase complex, which presumably promotes HY5 ubiquitination in the dark. Recently, RING-H2 proteins have been found to be associated with protoypical E3 ubiquitin ligases *in vivo*. *In vitro*, these RING-H2 components have been found to promote E2 ubiquitin conjugating enzyme dependent ubiquitination of respective target proteins. A RING-H2 protein, CIP8, has previously been characterized as an interaction partner for the RING finger domain of COP1. We thus tested, whether CIP8 can act in a similar E2 dependent fashion in promoting ubiquitin attachment to HY5 *in vitro*. This is indeed the case, and detailed biochemical evidence to support a role of CIP8 in HY5 ubiquitination will be presented.

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11 Arabidopsis TOUSLED protein kinase function in nuclear events.

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The *TOUSLED*(*TSL*) gene encodes a nuclear serine/threonine protein kinase, which is essential for the proper morphogenesis of leaves and flowers in Arabidopsis thaliana. Mutations at the *TSL* locus cause abnormal leaf and flower development. TSL is a member of a highly conserved TOUSLED-like kinase (TLK) family present in plants and animals. In humans, Tlk1 and Tlk2 are S-phase nuclear protein kinases that may function during DNA replication [Sillje, H. H. W., Takahashi, K., Tanaka, K., Van Houwe, G., and Nigg, E. A., EMBO J., 18, 5691-5702 (1999)]. In mouse, subcellular localization of mTlk2 changes during the cell cycle [Zhang, S., Xing, H., and Muslin, A. J., J. Biol. Chem., 274, 24865-24872 (1999)]. In Arabidopsis, a mitotic marker gene (CDB-GUS) shows an abnormal expression pattern in *tsl* mutants compared to wild type. To elucidate the putative role of *TSL* during plant cell division, we determined the expression of TSL at the protein and transcript level in synchronized Arabidopsis cell suspension cultures and susbsequently its autophosphorylation activity and activity towards the substrate MBP. The protein and transcript level remain at similar levels throughout the cell cycle, but the specific activity of the protein kinase changes during various stages of the cycle. Also, in G2/M, a lower mobility form of TSL appears, which then disappears in G1 and S. We have attempted to find possible substrates and interacting proteins in Arabidopsis cells, and the preliminary analysis from these experiments will be discussed, including the description of a myb-family DNA binding protein which interacts with TSL in the two-hybrid system.

12 TORMOZ is required for orientating cell division planes in early embryo development

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Precisely orientated cell division planes is a mark of early embryogenesis in Arabidopsis. However, relatively few plant genes have been identified to be involved with this process. Recently, we isolated a mutant named *tormoz*(*toz-1*) from our *Ds*gene trap collection that segregates for arrested embryos which show abnormal planes of cell division in the embryo proper (EP), but not in the suspensor. The zygote of mutant embryos elongates and divides transversely as in wild type, but further cell division planes of the EP are disrupted. Unlike wild type, all cells in the mutant EP have the potential to divide longitudinally, transversely or occasionally obliquely. Thus, the plane of division that each cell adopts bears little relationship to the orientation of the previous division, or that of the neighboring cells. In addition, embryo arrest may occur at any time, with the most advanced mutant embryos consisting of about 32 cells.

The gene disrupted in the *toz-1*mutant is predicted to encode a WD-40 repeat containing protein with unknown function. Through RT-PCR analysis we found that it is not a null mutation. Detailed observations of heterozygous plants using the GUS fusion from the *Ds*gene trap show that *TOZ*is expressed in tissues undergoing rapid growth and localizes to the nucleus. A uniquely conserved gene homologous with *TOZ*is found in many Eukaryotic species including yeast, insects and humans. Using the *TOZ*homologue in fission yeast we found that septum formation is not perturbed in null mutants, but disruptions to nuclear integrity and chromosome structure are observed during cell division. It is therefore likely that *TOZ*and its homologues perform an essential function during cell division in Eukaryotes. The *toz-1*mutant affects divisions of the EP but not the suspensor suggesting that an important aspect of TOZ function is to direct longitudinal cell divisions in the Arabidopsis embryo.

13 Molecular analysis of *FVE* and *PRE*: two genes involved in the autonomous flowering promotion pathway

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The transition from the vegetative to the reproductive phase of a plant is a complex process controlled by multiple environmental and endogenous factors. The genetic and molecular dissection of this developmental switch in Arabidopsis has led to the involvement of at least two main flowering promotion pathways: i) the photoperiod promotion pathway, primarily involved in the photoperiodic induction of flowering and ii) the autonomous flowering promotion pathway (AFPP). Mutants in the AFPP show altered flowering phenotypes independently of the photoperiod in which plants grow, and therefore, it is speculated they might identify genes encoding central molecular elements controlling this process. In the present work we have analysed two mutants involved in the AFPP: i) the late flowering mutant *fve*, and ii) *precocious* (*pre*), an early flowering mutant largely suppressing the late flowering of *fve*. *FVE* has been located in the middle genomic region of chromosome 2, while *PRE* has been assigned to the bottom part of chromosome 1. Using map-based strategies we have isolated both genes, thus providing new molecular pieces that participate in the flowering induction process.

14 Genomic analysis of the floral transition

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Functional genomic analysis of the Arabidopsis transcription factor complement reveals many previously unrecognized regulators of the floral transition. For example, as part of this study, we identified five additional MADS box factors with a very high degree of similarity to FLOWERING LOCUS C (FLC), a protein known to be a core regulator of the vernalization response. Genetic and expression analysis of these additional factors suggests that they likely have a related role to FLC in the control of flowering time. The large number of transcription factors influencing flowering, indicates that a significant proportion of plant genomes might directly or indirectly affect the floral transition. Thus, a true understanding of this crucial decision now requires genome wide analyses to be used in conjunction with the traditional experimental approaches that focus on individual or small groups of genes.

15 The role of the Arabidopsis copper transporter RAN1 in the biogenesis of ethylene receptors

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The characterization of Arabidopsis mutants displaying ethylene insensitivity led to the identification a small family of proteins consisting of ETR1, ETR2, ERS1, ERS2, and EIN4. Expression of ETR1 and ERS1 in the yeast *S. cerevisiae* showed they possess ethylene-binding activity, supporting their role as ethylene receptors. Biochemical studies indicated that the ethylene-binding domain of ETR1 is contained within the first 128 residues of ETR1. This region shows the highest degree of sequence identity between all the members of the family. Despite the fact that sequence analysis of ETR1 did not identify any metal-binding motifs, copper ions enhance ethylene binding *in vitro*. Furthermore, copper ions co-purified with the ethylene-binding domain of at a stoichiometry of one copper per ETR1 dimer. This indicates that ETR1 and its homologues bind ethylene using a novel metal-binding motif. We are interested in identifying the requirements for the biogenesis of such a unique ethylene-sensing copper-binding motif. A possible role for the copper-homeostasis machinery of *Arabidopsis* in ethylene perception/signaling is supported by the ethylene-response phenotype of seedlings with mutations in *RAN1* (response to antagonist). RAN1 has sequence similarity to the CPX-type of copper-transporting ATPases typified by the Menkes/Wilson disease proteins from humans and Ccc2p from *S. cerevisiae*. To test the hypothesis that RAN1 delivers copper ions to the ethylene receptors, we examined the biogenesis of ETR1 in *S. cerevisiae* mutants lacking Ccc2p (Δccc2+ETR1). The results showed that Ccc2p is essential for the biogenesis of heterologous ETR1 in *S. cerevisiae*. Addition of copper or expression of RAN1 from *Arabidopsis* restored ethylene binding in Δccc2+ETR1 yeast. We have further confirmed the role of RAN1 in the biogenesis of ethylene receptors analyzing ethylene binding in *Arabidopsis* seedlings carrying *ran1* weak and strong alleles. A similar analysis showed that CCH1 (copper chaperone 1) is not involved in the biogenesis of ethylene receptors *in planta* . We present a model for the mode of action of the different *ran1* alleles in ethylene responses.

16 BIK1, a potential partner for BRI1 in mediating brassinosteroid signaling

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Brassinosteroids (BRs) are the only class of plant steroids that play important roles throughout the plant life cycle. Genetic screens in *Arabidopsis* for BR-signaling mutants identified BRI1, a leucine-rich-repeat (LRR) receptor-like kinase, as an essential component in BR signaling. Several recent biochemical and molecular studies confirmed that BRI1 is a critical component of a membrane BR receptor. To identify other components of this membrane receptor complex, we performed a yeast two-hybrid screening using the BRI1 kinase domain as a bait and identified several BRI1-specific interactors. One of them is an LRR-containing receptor-like kinase that was named as BRI1-Interacting receptor-like Kinase 1 (BIK1). When coexpressed in yeast cells, a full-length BIK1 protein can be co-immunoprecipitated with a full-length BRI1 protein. GUS staining with *BIK1-GUS* transgenic plants indicated that *BIK1* is expressed mainly in young root tips and expanding young leaves, while BIK1:GFP fusion protein analysis showed that BIK1 is localized on the plasma membrane. Transgenic plants overexpressing *BIK1* gene showed narrow leaves with elongated petiole, reminiscent of phenotypes of *BRI1* overexpressing lines. In contrast, a T-DNA inserted *bik1* knockout mutant displayed compact rosette with rounded leaves and short petioles, resembling weak *bri1* mutants. Thus, BIK1 might function as a partner for BRI1 to mediate BR signaling critical for plant growth. Further studies demonstrating a direct interaction between BIK1 and BRI1 in plant cells are in progress.

17 Auxin Action Requires SCF^{TIR1}-Dependent Degradation of the Aux/IAA Proteins

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Genetic and biochemical studies have shown that auxin action depends upon the function of a ubiquitin-protein ligase (E3) called SCF^{TIR1}. On the basis of these results we have proposed that auxin-response depends on SCF^{TIR1}-degradation of one or more negative regulators. The Aux/IAA genes encode nuclear proteins with very short half-lives. These proteins appear to influence auxin-regulated gene expression, possibly by interacting with members of the ARF family of transcriptional regulators. Remarkably, dominant mutations in five members of the Aux/IAA gene family; *AXR2/IAA7, AXR3/IAA17, SHY2/IAA3, SLR/IAA14,* and *IAA28* all result in auxin-related defects. In every case the mutation results in an amino acid substitution within domain II of the protein. Several studies have shown that these mutations acts to stabilize the proteins (Worley et al., 2000; Quellet et al., 2001). To investigate the relationship between SCF^{TIR1} and the Aux/IAA proteins, we have performed a series of experiments with the AXR2 protein. An AXR2 antibody was used to show that AXR2 is more abundant in the *axr2-1* mutant, consistent with the proposed stabilizing effect of mutations in domain II of the protein. In addition, AXR2 is more abundant in the *tir-1* and *axr1-12* mutants compared to wild type, suggesting that SCF^{TIR1} regulates AXR2 degradation. To test for interaction with SCF^{TIR1}, purified GST-AXR2 was used to recover interacting proteins from plant extracts. SCF^{TIR1} was recovered together with GST-AXR2, demonstrating that AXR2 is associated with the SCF complex. Addition of ubiquitin-activating enzyme (E1) and conjugating enzyme (E2) to the GST pulldown resulted in ubiquitination of AXR2. In contrast, a version of AXR2 identical to that encoded by the *axr2-1* mutant gene did not interact with SCF^{TIR1} indicating that domain II of the protein includes residues that are required for interaction with SCF^{TIR1}. It is likely that the dominant mutations in the other *Aux/IAA* genes also stabilize the respective proteins by preventing interaction with SCFs. These results indicate that AXR2, and probably other members of the Aux/IAA family are substrates for SCF^{TIR1} and related SCF complexes. We propose a model in which auxin regulates transcription of downstream genes by promoting the degradation of the Aux/IAA proteins.

18 Cytokinin signaling in Arabidopsis thaliana requires the 26S proteasome subunit RPN12

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The 26S proteasome is essential for many aspects of eukaryotic cell growth and development by degrading important cell regulators, especially those conjugated with multiple ubiquitins. Bound on both ends of the 20S core protease (CP) is a multisubunit regulatory particle (RP) that plays a crucial role in substrate selection by a yet unknown mechanism(s). Here, we show that the RPN12 subunit of the *Arabidopsis* RP is involved in cytokinin signaling. A T-DNA mutant of RPN12a displays all the hallmarks of a cytokinin response defect, including a decreased rate of leaf formation, delayed skotomorphogenesis and decreased sensitivity to exogenous cytokinins. The cytokinin-inducible genes *CycD3* and *NIA1* are constitutively upregulated in *rpn12a-1* suggesting a disruption of feedback-inhibition control. *Rpn12a-1* showed decreased auxin sensitivity as well, further illustrating the close interaction between auxin and cytokinin regulation. T-DNA insertion mutations affecting the RP subunits RPN10 and RPN5a did not result in decreased cytokinin sensitivity, indicating that different subunits of the RP are involved in different regulatory processes. In yeast, RPN12 is necessary for the G1/S and G2/M transitions of the cell cycle, phases that have been shown to be under cytokinin control in plants. Collectively, the data suggest that RPN12a as part of the *Arabidopsis* 26S proteasome controls cytokinin signaling by specifically affecting the stability of one or more regulatory factors involved in cell division.

19 *CHROMOMETHYLASE3* is required for maintenance of CpXpG methylation.

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Cytosine methylation plays a major role in determining the epigenetic expression state of eukaryotic genes. This methylation is most often found at the symmetrical dinucleotide CG (or CpG sites). CpG methylation is maintained by the well-studied DNMT1 subfamily of methyltransferases, which includes *Arabidopsis* MET1. Methylation at sites other than CpG is also found in many organisms, but the mechanism by which this methylation is maintained is poorly understood. For unknown reasons, the floral development gene *SUPERMAN (SUP)* is prone to ectopic hypermethylation and gene silencing. These epigenetic silenced SUP alleles (called the *clark kent* alleles) are associated with dense hypermethylation at non-canonical cytosines (CpXpG and asymmetric sites). We performed a genetic screen for suppressors of a hypermethylated clark kent mutant, which identified nine loss-of-function alleles of *CHROMOMETHYLASE3(CMT3)*, a novel cytosine methyltransferase homolog. These *cmt3* mutants display a wild-type morphology, but exhibit decreased CpXpG methylation of the *SUP* gene and of other sequences throughout the genome. They also show reactivated expression of endogenous retrotransposon sequences. These results show that a non-CpG DNA methyltransferase is responsible for maintaining epigenetic gene silencing.

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20 Destabilization of Arabidopsis genome by *ddm1* (*decrease in DNA methylation1*) mutation

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Transposable elements potentially cause extensive genome rearrangements. Methylation of cytosine residues has been proposed to be a mechanism to suppress the transposons, but the experimental evidence has been limited. Reduced methylation of repeat sequences results from Arabidopsis mutations in the *DDM1* (*decrease in DNA methylation*) gene, which encodes a protein similar to the chromatin remodeling factor SWI2/SNF2 (ref 1). A striking feature of the *ddm1* mutation is that it induces a variety of developmental abnormalities by causing heritable changes in other loci. One of the *ddm1*-induced abnormalities, *clam*, was caused by insertion of *CAC1*, a novel endogenous Arabidopsis transposable element. This class of Arabidopsis elements transposed and increased in copy number at high frequency specifically in the *ddm1* hypomethylation background. Thus the *DDM1* gene not only epigenetically ensures proper gene expression (ref 2), but also stabilizes the genome structure by controlling transposon behavior.

1. Jeddeloh et al. (1999) *Nat. Genet.* 22, 94-97.
2. Soppe et al. (2000) *Molecular Cell* 6, 791-802.

21 *BRU*: a gene linking DNA damage responses and transcriptional gene silencing

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From a T-DNA mutagenized *Arabidopsis* collection, we have identified a mutant with elevated sensitivity to DNA damaging agents such as methyl methane sulfonate (MMS), mitomycine C (MMC) and UV-C. The mutant is also altered in the branching pattern that causes brushy like phenotype (hence the name 'bru'). In addition, bru has flat and fasciated stems. By crossing bru to marker line for homologous recombination (Swoboda et al., 1994), 4-5 fold increase of intrachromosomal recombination was observed for bru compared to wild type plants. Transcriptionally silent information (TSI)is an endogenous marker for transcriptional gene silencing (TGS)(Steimer et al., 2000). TSI is expressed in TGS mutants but not in wt plants. Surprisingly, TSI is also expressed in bru; hence, bru releases TGS. The methylation level of the 180 bp pericentromeric repeat is, however, not altered in bru. Since T-DNA insertion was not linked to the bru mutation, BRU gene was mapped using cleaved amplified polymorphic sequence (CAPS) and simple sequence length polymorphism (SSLP) markers. The BRU gene structure will be discussed at the meeting. BRU is the first Arabidopsis gene linking in two processes: DNA damage response and transcriptional gene silencing.

Steimer A., Amedeo P., Afsar K., Franz P., Mittelsten Scheid O., Paszkowski J. (2000). Endogenous targets of Transcriptional gene silencing in *Arabidospsis*. Plant Cell 12:1165-1178. Swoboda P., Gal S., Hohn B., Puchta H., (1994) Intrachromosomal homologous recombination in whole plants. EMBO J. 13:484-489.

22 Plant components involved in homologous recombination: A mutant approach

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Homologous recombination is an important process both in meiosis and during somatic development. It is striking that this process is involved both in genome flexibility, which is important for evolution, and genome stability by participating in DNA repair processes. Although it was clearly shown that a variety of environmental factors affect the frequency of somatic recombination events, there is rather poor knowledge about the regulatory cascades involved and their connections to the recombination process. To address this question, we used a mutant approach as a powerful tool. We wanted to look more specifically for plant components involved in the regulatory pathways related to homologous recombination and in the recombination process itself. Using homologous recombination as a marker for genome dynamic, we established and screened a mutant collection of *Arabidopsis thaliana* ecotype Columbia for altered recombination phenotypes in the absence of external stresses. Monitoring somatic recombination phenotypes directly *in vivo* is one of the key point for such approach. For this, we used a luciferase reporter line designed for detection of intrachromosomal recombination harbouring two truncated but partially overlapping segments of the luciferase gene in opposite orientation. This reporter line was then mutagenised by T-DNA activation tagging, giving rise to a collection of about 20000 independent transformants which were directly screened for dominant recombination-up phenotypes. This first round of screening yielded 30 candidates with a significant increase in the number of luciferase sectors ranging from 10 to more than 100 fold. Although in most of the cases the candidate plants and their progeny seem not be developmentaly affected, several plants displayed dramatic effects ranging from bushy phenotypes to sterile or non viable plants.The genetic characterisation of the mutations and the molecular analysis of the putatively activated genes are presented. The knowledge brought by these results might give new powerful insights into the relation between the genome and it's environment

23 Genetic variation in *Arabidopsis thaliana* and related species

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The genus *Arabidopsis* contains 10 species from Eurasia and North America. These species are genetically diverged for many aspects of morphology, physiology, breeding system, and cytology. Information from *A. thaliana* can be applied to close diploid relatives to address questions in functional and evolutionary genomics. Genetic changes during speciation and adaptation to environmental conditions will be discussed.

Within the species *A. thaliana* there is extensive nucleotide polymorphism among ecotypes. We quantified polymorphism from 400 loci in a diverse sample of ecotypes. These data show strong support for recent population expansion, and potential for linkage disequilibrium mapping of functionally important natural variation. Genetic variation in *A. thaliana* is influenced by Pleistocene history, adaptation to local environmental conditions, human disturbance, and population expansion.

24 Of flowers, branches and TCP genes

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The TCP genes code for proteins containing a predicted non-canonical basic helix-loop helix domain thought to be involved in transcriptional regulation: the TCP domain.

Two members of the TCP gene family, *CYCLOIDEA* (*CYC*) and *TEOSINTE BRANCHED 1* (*TB1*), have been implicated in the evolution of key morphological traits. The *CYC* gene, from *Antirrhinum*, is involved in the control of floral dorsoventral asymmetry, a trait evolved relatively late in the history of angiosperms. The *TB1* gene controls developmental switches that contributed to the evolution of maize from its wild ancestor teosinte. Interestingly, both genes affect axillary structures, flowers in the case of *CYC*, lateral buds in the case of *TB1*. Are *CYC* and *TB1* ortholog genes? How were the ancestral *CYC* and *TB1* genes recruited to carry out these new functions? What was their ancestral role and what made them suitable to generate new morphological traits? .

We are trying to address these questions in *Arabidopsis*, a species distantly related both from *Antirrhinum* and maize. *Arabidopsis* has a weak apical dominance -one of the traits controlled by *TB1*- compared to maize, and *Arabidopsis* flowers have no obvious dorsoventral asymmetry. Therefore, we may find, in this species, the ancestral version of *CYC*/*TB1* before they were co-opted to carry out their new roles.

We have identified several *CYC*/*TB1*-like genes and we are currently studying their function by genetic and expression analyses. Moreover we have identified the complete *Arabidopsis* TCP family, formed by 24 members that map in all five chromosomes. TCP genes fall in two subclasses, one related to *CYC*/*TB1* and another one related to the PCFs, rice transcription factors that bind to the promoter of the Proliferating Cell Nuclear Antigen gene. The evolutionary history of *CYC*/*TB1* has to be placed in the context of the evolution of this gene family. Phylogenetic and functional analysis of the other TCP genes is helping us clarify the picture of the evolution and biological function of *CYC* and *TB1* in angiosperms.

25 The effect of R-gene resistance versus susceptibility on infected plants' fitness

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Recent molecular evolutionary analyses of R-gene polymorphisms indicate that susceptible, null R-gene alleles have been maintained in natural plant populations by selection for very long time periods. This suggests that there is a cost to R-gene resistance. Costs of resistance are classically thought to involve a reduction in the fitness of resistant individuals in the absence of enemies. However, another possibility is that resistant individuals are less fit than susceptible individuals *when pathogens are present* in some cases. For example, tolerance might be more effective than a metabolically costly HR and SAR when dealing with a weak pathogen. We investigated the fitness effects of resistance and susceptibility at RPS2 in *Arabidopsis* in the presence and absence of intraspecific competition. Columbia wild type (R) and Columbia rps2 (S) mutant plants were infected with *Pseudomonas syringae* pv. tomato DC3000 containing avrRpt2, and their fitness measured. When grown in the absence of competition, RPS2 resistant plants had a lower total seed mass than RPS2 susceptible plants. In the presence of competition, RPS2 resistant plants had a higher total seed mass than RPS2 susceptible plants. Control resistant and susceptible plants infected with only a mock treatment showed no difference in total seed mass, suggesting that the resistant and susceptible responses to disease are responsible for the differences in fitness. We will present evidence that a phenological response to infection by the susceptible plants partly accounts for the fitness differences observed.

26 Molecular Evolution of the Receptor-Like Kinase Family in Arabidopsis

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Signal perception through cell surface receptors is one of the primary mechanisms for living organisms to sense stimuli and changes in their biotic/abiotic environments. In plants, one of the most abundant classes of cell surface receptor is the receptor-like kinases (RLKs) with a predicted signal sequence, single transmembrane region, and cytoplasmic kinase domain. To provide a framework for understanding the function and evolution of RLKs, an analysis of all RLKs genes in the Arabidopsis genome was conducted. We found that RLKs belong to a large gene family with more than 610 members, representing 59% of the total ser/thr/tyr kinases and nearly 2.5% of protein coding genes in Arabidopsis. Based on the phylogeny of kinase domain sequences, the presence or identity of the extracellular domains, and the intron-exon organizations of all 610 members, RLKs can be classified into at least 35 subfamilies. The members of each subfamily have similar extracellular domains, suggesting that a single domain fusion event contributed to the founding of each subfamily. Surprisingly, this structurally defined group of genes is monophyletic with respect to kinase domains when compared to the other eukaryotic kinase families. This indicates that Arabidopsis RLKs have a single origin. Moreover, Raf kinases, plant RLKs, and animal receptor tyrosine kinases form a very well supported group distinct from other kinases in the eukaryotic kinase superfamily, indicating that a single kinase family was ancestral to receptor kinases in both plants and animals. Interestingly, we found that Pelle kinases, with merely 5 members in the genomes of fly, worm, and human combined, are the metazoan homologs of plant RLKs. In addition, a survey of available EST records reveals that mosses, ferns, conifers, monocots and dicots have similar percentages of ESTs representing RLK/Pelle homologs, suggesting that the size of this gene family may have been close to the present day level before the diversification of land plant lineages. Based on the distribution patterns of RLKs on the chromosomes and the kinase phylogeny, we found that tandem and large-scale duplications are two of the major mechanisms contributing to the expansion of this gene family in Arabidopsis.

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27 Epigenetic regulation of orthologous genes in Arabidopsis polyploids

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The fate of redundant genes resulting from genome duplication is poorly understood. Previous studies indicated that ribosomal RNA genes from one parental origin are epigenetically silenced during interspecific hybridization or polyploidization. Regulatory mechanisms for protein-coding genes in polyploid genomes are unknown, partly due to difficulty in studying expression patterns of homologous genes. Here we apply AFLP-cDNA display to perform a genome-wide screen for orthologous genes silenced in *Arabidopsis suecica*, an allotetraploid derived from *Arabidopsis thaliana* and *Cardaminopsis arenosa* . We identified ten genes that are silenced from either *A. thaliana* or *C. arenosa* origin in *A. suecica* and located in four of the five *A. thaliana* chromosomes. These genes represent a variety of RNA and predicted proteins including four transcription factors such as TCP3. The silenced genes in the vicinity of *TCP3* are hypermethylated and reactivated by blocking DNA methylation, suggesting epigenetic regulation is involved in the expression of orthologous genes in polyploid genomes. Compared to classic genetic mutations, epigenetic regulation may be advantageous for selection and adaptation of polyploid species during evolution and development.

28 NBS-LRR *R* gene products: Functional domains and divergent downstream transcript profiles.

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We are investigating the molecular basis of resistance gene (*R* gene) function and the signal transduction events that activate gene-for-gene resistance, HR cell death and other forms of disease resistance. Working with Arabidopsis *RPS2*, we have investigated the roles of the nucleotide binding site (NBS) and the leucine-rich repeat (LRR). Our evidence for nucleotide binding by recombinant RPS2 protein will be presented, along with a functional dissection of allowable amino acid changes in the NBS of RPS2. In separate studies, we have found that the LRR domain not only determines pathogen specificity, but can also control effective interaction with other host factors required for resistance. In a third set of studies, Arabidopsis responses mediated by four different *R*/*avr* pairings are being compared by expression profiling using Affymetrix GeneChips. Expression profiling experiments were replicated directly or with variation. For direct replication, essentially identical experiments were performed on two separate dates using *P. syringae* pv. *tomato* DC3000 to deliver Avr signal. For our first "variation" performed to date, the defense-eliciting Avr signal was delivered to plants by inoculation with non-virulent but *hrp*-secretion-competent *P. syringae* pv. *glycinea*. These experiments are revealing reliable and less reliable technical aspects of expression profiling experiments, but are also revealing common and distinctive aspects of the plant responses elicited during different *R*/*avr* interactions.

29 Function of RPM1 requires RIN4, a virulence target guarded by RPM1

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Supported by NSF grant IBN-9724075 to JLD *D. M. is a DOE fellow of the Life Sciences Research Foundation

RPM1 is an R-gene that confers resistance against Pseudomonas syringae carrying the avirulence (avr) genes avrB or avrRpm1. We have discovered an unknown Rpm1 interacting protein (Rin4) which interacts, in yeast and in vivo, with both Rpm1 and AvrB. Rin4 is required for function of Rpm1. Suppression of Rin4 protein-levels by antisense RNA inhibits the hypersensitive response (HR) normally induced by RPM1. In fact, suppression of Rin4 protein-levels causes Rpm1 protein-levels to decline. Significantly, suppression of Rin4 does not affect the HR induced by Rps2 in response to AvrRpt2. Rps2 is structurally very similar to Rpm1 and several mutants have been isolated which influence the function of both proteins. Rin4 functions prior to the convergence of these pathways and is specific to Rpm1. The guard hypothesis predicts that an R-protein exists in a complex with a partner protein that is the virulence target of an Avr-protein. Rpm1 is in a complex with Rin4 in uninfected tissue. When expressed in tissue lacking Rpm1, AvrB and AvrRpm1 enter into a complex with Rin4. AvrB and AvrRpm1 also induce a mobility shift in Rin4. Expression of AvrRpt2 induces rapid degradation of Rin4. These manipulations of Rin4 by Avr-proteins are likely to underlie the virulence activity of these proteins. Consistent with its manipulation by virulence factors, Rin4 is a negative regulator of defense. Reduced levels of Rin4 cause increased resistance against normally compatible isolates of Peronospora parasitica and Pseudomonas syringae. Rpm1 "guards" Rin4 from manipulation by AvrB and AvrRpm1.

30 Role of Atrboh in Defense Response and Programmed Cell Death

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Different mechanisms have been hypothesized as a putative source of reactive oxygen intermediates (ROI) observed early in the plant defense response. However, the ultimate origin of this ROI remains elusive. Our goal is to address, using functional genomics tools, the functions of the *Atrboh*, the *Arabidopsis* NADPH oxidase *gp91*^{phox} gene family. The hypothesis to be tested is that members of this gene family control ROI production during defense response and in several developmental contexts. We analyzed mutant lines in the two highest expressed *Atrboh* genes *D* and *F*. Specific stains for ROI show that *AtrbohD* and *F* are responsible for most of the ROI observed during incompatible interactions with the bacteria *Pseudomonas syringae* and the oomycete pathogen *Peronospora parasitica*. Whereas defense response is not greatly affected in these mutant lines, they display altered cell death when compared to the wild type. A decrease in electrolytic leakage is observed in these lines after *P. syringae* DC3000 (*avrRpm1*) inoculation. An enhanced cell death phenotype occurs after infection of the mutant lines with a *P. parasitica* race that displays partial resistance. Paradoxically, although *AtrbohD* contribution to total ROI production is greater than *AtrbohF*, individual mutant *Atrbohf* displays strongest effect on cell death.

Funded by NIH grant RO1 GM57171-03 and NSF grant IBN-0077887 to JLD.

31 Specificity in The Glucosinolate/Myrosinase System Regulates Defense Against Insect Herbivores

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Glucosinolates are naturally occurring thioglycosides that are hydrolyzed upon tissue damage by the endogenous thioglucosidase, myrosinase. Hydrolysis of glucosinolates yields an unstable aglycone intermediate, which is spontaneously or enzymatically converted into various toxic compounds (isothiocynates, cyanoepithioalkanes or nitriles). Their effects on insect herbivory have been widely studied.

We analyzed several mapping populations for QTLs that regulate glucosinolate/myrosinase amount, glucosinolate/myrosinase type and resistance against generalist, *Trichoplusia ni*, and specialist, *Plutella xylostella*, lepidopteran herbivores. Comparing QTLs for herbivore resistance versus QTLs for the glucosinolate/myrosinase system showed that *Trichoplusia ni* herbivory is deterred by glucosinolate amount and the type of breakdown product formed. In contrast, *Plutella xylostella* herbivory is not altered by variation in the glucosinolate/myrosinase system.

Utilizing a combination of quantitative genetics, Mendelian genetics and enzymology, we have identified the genes underlying several QTLs. These genes encode enzymes that specify the amount and type of glucosinolates and the nature of their breakdown products. The identity and characterization of these genes will be presented.

32 *Rar1* - a component of disease resistance signaling in Arabidopsis

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To identify further genes involved in *RPP5*-mediated resistance signaling in Arabidopsis, fast neutron- and EMS-mutagenized populations of Landsberg *erecta* were screened for loss of resistance to the oomycete pathogen *Peronospora parasitica*, isolate Noco2. One mutant, designated *rpr2* (required for *RPP5* resistance), was among those selected for further study since it was shown to be non-allelic to previously identified components of *RPP5* gene-mediated resistance (*EDS1* and *PAD4*). Positional cloning of *RPR2* was undertaken, and it was found to be the Arabidopsis orthologue of barley *Rar1*, a gene originally characterized as an essential component of *Mla12*-specified resistance to the powdery mildew fungus. Previous analysis of the barley Rar1 protein sequence has revealed two zinc binding (CHORD) domains that are highly conserved in plants and animals. Extended sequence comparisons suggested a possible role of Rar1 in ubiquitination of proteins.

Pathogenicity tests have shown *AtRar1* is not only required for *RPP5*-mediated resistance, but also for the function of a range of structurally different *R* genes, including those required for resistance to the bacterial pathogen *Pseudomonas syringae*. Consistent with the *rar1* mutant phenotype in barley, *Atrar1* mutants were compromised in pathogen-triggered whole-cell hydrogen peroxide accumulation and cell death, suggesting that susceptibility of *Atrar1* mutants coincides with the inability to mount a host cell death response.

33 Analysis of the *Arabidopsis Sgt1* Mutant Suggests a Link Between Ubiquitin Mediated Protein Degradation and Plant Disease Resistance

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An EMS mutagenized population of *Arabidopsis thaliana*, ecotype Lansberg *erecta* (La-*er*), was screened for loss of *RPP5*-mediated resistance to the obligate biotroph *Peronospora parasitica* isolate Noco2. A number of mutant lines were isolated. A positional cloning strategy was employed to help identify one of these (*rpr1-1*: required for *RPP5* resistance). Analysis of recombinants derived from a *rpr1-1* La-*er* x Columbia mapping population located the mutant allele to a region of approximately 50kb on the lower arm of chromosome IV. Direct sequence analysis of predicted ORFs within this region highlighted a G to A transition, causing the formation of a premature stop codon in one particular ORF. The sequencing of three other independent *rpr1-1* alleles confirmed that *RPR1* was the *Arabidopsis* homolog of the yeast gene *SGT1*. Yeast SGT1 is a key component of the SCF (Skp1p/Cdc53p/F box protein) ubiquitin ligase complex which targets proteins for proteosome mediated degradation. SGT1 is also required for the formation of the yeast kinetochore complex CBF3.

AtSgt1 is required by a discrete subset of *R*-gene mediated pathways including those from a number of other *P. parasitica* isolates. Pathogenicity assays using a *Pseudomonas syringae* strain expressing different avirulence genes suggest that bacterial pathogen recognition pathways do not require *AtSgt1*. These data suggest that *R*-gene mediated pathways are not linear, but partially overlapping and much more complex than at first thought. This possibly confers a large degree of flexibility to the type and timing of the resistance response.

The mutant lines are disrupted in their ability to accumulate whole-cell H_2O_2 at the site of attempted pathogen attack. The inability to accumulate reactive oxygen intermediates is correlated with a loss of cell death (hypersensitive response) and an increase in pathogen growth. Analysis of pathogeneisis related (PR) genes in response to *P. parasitica* infection show that *AtSgt1* has delayed or reduced levels of *PR1* gene expression. The disease phenotype can be rescued by pre-treating with the salicylic acid analogue BTH. This suggests that *AtSgt1* acts upstream or independently of SA accumulation.

34 Triggering, Potentiation, and Consequences of the Arabidopsis HR

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Bacteria carrying *avrB*, but not those carrying *avrRpt2*, can elicit a hypersensitive response (HR) on *ndr1-1* mutant plants. Bacteria carrying either *avr* gene triggered hydrogen peroxide production by wild type Columbia plants as assessed using a fluorescence-based *in vivo* assay. Hydrogen peroxide accumulation was first evident 4 hours post-inoculation with either strain. However, levels increased more rapidly in Columbia plants inoculated with bacteria carrying *avrB* than in those inoculated with bacteria carrying *avrRpt2*. Bacteria carrying *avrRpt2* elicited no detectable hydrogen peroxide production by *ndr1-1* plants. Bacteria carrying *avrB* elicited a response that was similar to that of Columbia in kinetics but of lesser intensity at early time points. These data, considered together with strong evidence that the direct effects of the *ndr1* mutations are to block salicylic acid production induced by either bacterial strain to a comparable extent, leads us to conclude that high level hydrogen peroxide production is a consequence, not a cause of the HR. Further, these data lead us to predict that a cell death ?riggering factor?is produced in response to avirulent bacteria, that this factor is produced at a higher level in response to bacteria carrying *avrB*, and that salicylic acid potentiates the action of this factor. Analysis of a *ndr1-1*/*npr1-2* double mutant showed that *ndr1-1* was epistatic to *npr1-2* with respect to HR phenotypes, suggesting that the documented *NPR1*-dependent negative feedback loop does not control the HR. Epistasis analysis also demonstrated that cell death makes a *NPR1*-independent contribution to PR gene induction.

35 Genetic screen to identify hdn Arabidopsis mutants: HR Despite NOS Inhibitor.

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Key words: HR, NOS, nitric oxide, PCD, HDN

12,751 EMS-mutagenized M2 Arabidopsis were screened for their ability to overcome an L-NNA (nitric oxide synthase inhibitor) block of hypersensitive response (HR) elicited by pseudomonas. 793 plants tested positive. We re-screened 15 M3 from each positive and identified 90 lines which are reproducibly positive. We propose to name them "hdn" for : HR Despite NOS Inhibitor. The screen was repeated on 14,888 activation tagged T2 plants, and 276 plants tested positive initially. We began preliminary characterization of these mutants with dip disease assays. 58 EMS mutants have been tested, and 22 lines have significantly different responses to pseudomonas compared to wild type. The responses range from enhanced resistance, to enhanced susceptibility, as well as severe spreading cell death. These changes in disease responses demonstrate the variety of different mutants isolated in this screen. EMS mutants were crossed to Landsberg erecta for mapping and positional cloning. They were also back crossed to wild type Columbia to test for dominance/recessivity, to perform Chi Squared analyses, and to reduce the mutational load. F2 populations are currently being analyzed. We expect to identify genes involved in nitric oxide signaling or parallel pathways which can bypass the requirement for nitric oxide in the plant pathogen defense response.

36 Characterization of transcription factors mediating COP1 regulated development.

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COP1, a putative E3 ubiquitin ligase, acts as a repressor of photomorphogenic development in the dark. So far, the only known target of COP1 mediated repression is the bZIP transcription factor HY5. In an effort to identify potential new COP1 targets, we performed a yeast two-hybrid screen using COP1 as bait. The screen identified five new proteins that specifically interact with the WD40 domain of COP1. One of these proteins is a homologue of HY5 (HYH) whereas three contain tandem repeated B-box Zn^{2+} finger motifs in their N-terminal portion, COP1 interacting CONSTANS-like (CCO), STO and a homologue of STO (STH). The fifth is a novel protein. Using a reverse genetic approach, we identified T-DNA insertions in both HYH and CCO. The insertions result in null mutations of *hyh* and *cco*. Plants with *hyh* and *cco* mutations flower early, similar to *hy5* and opposite to *co*. Both *hyh* and *cco* seedlings have elongated hypocotyls when grown in blue light, suggesting that HYH and CCO act as positive regulators of photomorphogenesis. Furthermore, both *hyh* and *cco* are able to suppress *cop1* phenotypes, providing evidence for a functional interaction. HYH is the closest HY5 homologue in arabidopsis, HYH is specifically degraded in the dark and we will present results suggesting that HYH can heterodimerize with HY5. To investigate the relationship between HYH and HY5, we made *hyh hy5* double mutants. The double mutant plants are paler than *hyh* and *hy5* plants, suggesting that HYH and HY5 act in concert to promote chlorophyll accumulation. A functional overlap between HYH and HY5 is further supported by micro array experiments showing a large overlap in the expression profiles of *hyh, hy5* and *hyh hy5* double mutant seedlings.In a separate line of experiments we found that seedling over expressing STH are greener than WT seedlings, suggesting that STH act in the same pathway as HY5. We will present results suggesting a mechanism for how STH can affect HY5 dependent transcription.

37 ELF3 Modulates Resetting of the Circadian Clock in Arabidopsis

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The Arabidopsis *early flowering 3-1* (*elf3*) mutation causes arrhythmic circadian output in continuous light, but there is some evidence of clock function in darkness. Here we show conclusively normal circadian function with no alteration of period length in *elf3* mutants in dark conditions and that the light-dependent arrhythmia observed in *elf3* mutants is pleiotropic on multiple outputs normally expressed at different times of day. Plants overexpressing ELF3 have an increased period length in both constant blue and red light; furthermore, etiolated ELF3-overexpressing seedlings exhibit a decreased acute CAB2 response after a red light pulse, whereas the null mutant is hypersensitive to acute induction. This finding suggests that ELF3 negatively regulates light input to both the clock and its outputs. To determine whether ELF3's action is phase dependent, we examined clock resetting by light pulses and constructed phase response curves. Absence of ELF3 activity causes a significant alteration of the phase response curve during the subjective night, and constitutive overexpression of ELF3 results in decreased sensitivity to the resetting stimulus, suggesting that ELF3 antagonizes light input to the clock during the night. The phase of ELF3 function correlates with its peak expression levels in the subjective night. ELF3 action, therefore, represents a mechanism by which the oscillator modulates light resetting. (BARD-US-2964-97; NIH: GM56006, GM07413; NSF: MCB-9808208, DGE-9552837)

38 PIF4, a bHLH protein acts as a negative regulator of phyB signaling in *Arabidopsis*

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The mechanisms by which the phytochrome (phy) family of sensory photoreceptors transduce light signals to control gene expression are unknown. Recently we have shown that a G-box-bound bHLH protein, PIF3, can interact with the biologically active Pfr form of phyA and phyB, thereby potentially providing a direct regulation of gene expression by these phytochromes. We have isolated and partially characterized another bHLH protein, PIF4 (*p*hytochrome *i*nteracting *f*actor 4), which also interacts with phytochrome. However, in contrast to PIF3, PIF4 interacts specifically with phyB, and does so selectively in its biologically active Pfr form. Missense mutants of phyB that are impaired in signaling show reduced binding to PIF4, suggesting a biologically relevant interaction. Overexpression of *PIF4* in transgenic *Arabidopsis* produces a hyposensitive phenotype specific to red light. Conversely, antisense *PIF4* lines show hypersensitive phenotype under red light. Interestingly, in a separate approach, we have isolated a hypersensitive mutant, *srl2*, from a genetic screen under continuous red light. *srl2* has a T-DNA insertion within the *PIF4* gene. The similar hypersensitive phenotype of the antisense lines and the T-DNA knockout mutant, and the converse hyposensitive phenotype of transgenic overexpression lines, indicate that PIF4 might act as a negative regulator of phyB signaling. The convergence of two independent approaches on the same factor provides compelling evidence that PIF4 functions in the early part of the phyB signaling pathway.

39 Molecular Genetics Analysis of Root Gravitropism and Polar Auxin Transport in Arabidopsis thaliana

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Shortly after gravistimulation, plant roots develop curvature at the distal elongation zone such that root tips are re-aligned with the new gravity vector in defined angles. This phenomenon (gravitropic response) is complex and involves several distinct processes. Substantial experimental evidence suggests that polar auxin transport play an important role in root gravitropic response. It is postulated that auxin (level or sensitivity) is asymmetrically distributed between the top and bottom sides of a gravistimulated root. The preferential accumulation of auxin at the bottom side results in auxin levels inhibitory for cell elongation, whereas cells on the top continue to elongate. Previously, we reported molecular cloning of the *Arabidopsis AGR1* gene. Our functional analyses indicated that AGR1 is a component of the auxin efflux carrier that plays a regulatory role in root gravitropism. The *Arabidopsis AGR1* gene (also known as *EIR1*, *PIN2* and *WAV6*) belongs to a small gene family. Using a functional genomics approach, we identified and isolated several *Agrobacterium* T-DNA introduced mutations in several members of the gene family. We will report phenotypic analysis and molecular characterization of these mutants.

40 *Arabidopsis* ZIG/SGR4, one of SNAREs, has a crucial role in the shoot gravitropism

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We have isolated a number of *Arabidopsis sgr (shoot gravitropism)* mutants with abnormal gravitropism to elucidate the molecular mechanism of gravitropism in higher plants. Genetic studies have suggested that the endodermal cells in which contain sedimenting amyloplasts are the gravity sensing cells.

Here, we report characteristics of *zig/sgr4* mutant and the responsible gene. *zig/sgr4* exhibited abnormal gravitropism in the hypocotyl and the inflorescence stem. Besides, its inflorescence stems elongated zigzag. Although the whole tissue patterning was basically normal in the inflorescence stem, the aberration of cell shape and size was observed in several tissues. In the endodermis of *zig/sgr4*, amyloplasts did not localize to the bottom of cells and many stayed on the opposite side both in the inflorescence and the hypocotyl.

ZIG encodes AtVTIa, a homolog of yeast v-SNARE Vti1p, suggested to be involved in vesicle transport to the vacuole. Then, the subcellular structures of the endodermis and the cortex were observed with electron microscopy. The amyloplasts were located on both top and bottom sides of large central vacuoles and appeared to stick to the cell periphery. Aberrant vacuolar/vesicular structures were occasionally found in the cytoplasm. Remarkable anomalies of vacuoles, fragmentation and vesiculation, were more frequently observed in the cortex cells. *ZIG* gene driven by endodermis specific *SCR* promoter (*pSCR::ZIG*) could complement the gravitropic response in *zig/sgr4* mutant. These results suggest that *ZIG* expressed in the endodermis is essential for gravitropism and that *ZIG* affects amyloplast localization probably through vacuolar function. Interestingly, *pSCR::ZIG* could not rescue morphological abnormalities observed in *zig/sgr4*. *ZIG* expressed in tissues other than the endodermis is responsible for the plant morphology presumably via vacuole biogenesis and function.

41 The Function of SGR2, a Novel Phospholipase-A1-like Protein, in Endodermal Cell Layer for Shoot Gravitropism

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In higher plants, shoots and roots basically grow upwards and downwards, respectively. To elucidate the molecular mechanism of the gravitropic response, we isolated many mutants showing abnormal shoot gravitropism in *Arabidopsis*. The inflorescence stem and hypocotyl of *sgr2* show abnormal gravitropic response, but the response of the root is normal. Moreover *sgr2* seedlings often show abnormality in shape and most of *sgr2* zygotes have large vacuoles on the apical side and divide at irregular positions, reported by us at the 10th conference. The *SGR2* gene encodes a novel protein with a lipase consensus sequence and one putative transmembrane domain, which is homologous to the phosphatidic acid-preferring phospholipase A1 in *Boss taurus*. The gene family has been reported only in eukaryote and *sgr2* is the first mutant found among the family. The endodermal cell layer is an essential tissue for the shoot gravitropism, containing sedimented amyloplasts to the gravity. In *sgr2* mutant, the endodermal cells were frequently irregular in size and shape and some amyloplasts did not localize at the bottom of the cells. To elucidate the *SGR2* function in endodermal cells, the *SGR2* gene was expressed only in endodermal cell of *sgr2-1* using the *SCR* promoter. The transgenic *sgr2-1* plant recovered the shoot gravitropism and the abnormality of the shoot endodermal cell. These results indicated that the *SGR2* in endodermal cell is essential for shoot gravitropism. To identify the subcellular localization of SGR2, the *SGR2-GFP*, *GFP-SGR2* under CaMV 35S promoter was induced in Arabidopsis culture cell and the fusion protein located in the vacuolar membranes or putative endosomes. Moreover, the *SGR2-GFP* gene driven by the native *SGR2* promoter recovered *sgr2-1* gravitripic phenotypes and GFP could be detected in the vacuoles in planta as soon as in culture cells. The results suggested that vacuolar membranes are involved in the early step of gravity perception in shoot, and SGR2 in vacuolar membranes is required for the step.

42 Genetic and Chemical Reductions in Protein Phosphatase Activity Alter Auxin Transport, Gravity Response and Lateral Root Growth

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Auxin transport is required for important growth and developmental processes in plants, including gravity response and lateral root growth. Several lines of evidence suggest that reversible protein phosphorylation regulates auxin transport. Arabidopsis *rcn1* mutant seedlings exhibit reduced protein phosphatase 2A activity and defects in differential cell elongation. Here we report that reduced phosphatase activity alters auxin transport and dependent physiological processes in the seedling root. Root basipetal transport is increased in *rcn1* or phosphatase inhibitor treated seedlings, but shows normal sensitivity to the auxin transport inhibitor, naphthylphthalamic acid (NPA). Phosphatase inhibition reduces root gravity response and delays the establishment of differential auxin-induced gene expression across a gravity-stimulated root tip. An NPA treatment that reduces basipetal transport in *rcn1* and cantharidin treated wild-type plants also restores a normal gravity response, indicating that increased basipetal auxin transport impedes gravitropism. Elevation of auxin transport in *rcn1* or phosphatase inhibitor treated seedlings does not require the *AGR1*/*EIR1*/*PIN2*/*WAV6* or *AUX1* gene products. In contrast to root basipetal transport, root acropetal transport is normal in phosphatase-inhibited seedlings in the absence of NPA, but shows reduced NPA sensitivity. Lateral root growth also exhibits reduced NPA sensitivity in *rcn1* seedlings, consistent with acropetal transport controlling lateral root growth. These results support the role of protein phosphorylation in regulating auxin transport, and suggest that the acropetal and basipetal auxin transport streams are differentially regulated.

43 Co-ordination of cell division and cell expansion during root development in *Arabidopsis thaliana*

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Since the cells of higher plants are non-motile, normal morphogenesis is ultimately dependent on the co-ordination of cell division and cell expansion. We are investigating the importance of localised co-ordination of cell division and expansion in determining the architecture of the *Arabidopsis thaliana* root. Using a GAL4-GFP transactivation system, the rate of cell division is altered in specific cell layers via targeted misexpression of cell cycle regulatory genes. The consequences of these perturbations are visualised using high-resolution confocal imaging, followed by computerised three-dimensional reconstruction of cellular arrangements.

Here we report on the effects of altering cell division rates specifically within the columella region of the root cap during embryonic development. We show that increasing or decreasing rates of cell division in the targeted cells results in concomitant changes in cell division rates and/or cell expansion in neighbouring cell layers, with little effect on overall root morphogenesis. This approach allows us to determine the range and direction of cell-to-cell communication and provides insights into how cell division and expansion are co-ordinated locally via intercellular signalling within the root meristem.

44 Possible target genes of the *ANGUSTIFOLIA* gene, a plant CtBP, that regulates polar elongation of leaf cells

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Focusing on mechanisms that govern polarized growth of leaves in *Arabidopsis*, we have found that the *AN* gene regulates width of leaves and that the *ROT3* gene regulates length of leaves (Tsukaya et al. 1994, Tsuge et al. 1996, Kim et al. 1998, 1999). The *AN* gene encodes a member of the CtBP family, which are known to act as a transcriptional repressors in animal kingdom. *AN* is the first member of CtBP family isolated from plants.

To identify the targets of the *AN* gene as a transcriptional regulator of the other genes, two-hybrid analysis and microarray analysis was carried out under the Monsanto Arabidopsis Microarray Program. As a result, it was found that the *an* mutant expresses some genes At a higher level than the wild type, suggesting that the *AN* gene might work as a repressor of such genes. These possible candidates of target genes of the AN protein include Zn-finger protein, MAP kinase, MYB- and MYC-like protein. Interestingly, one of EXGT gene family that is thought to regulate loosening of cell wall also was found to be specifically up-regulated in the *an* mutant. Based on the results from analyses of the gene, molecular mechanisms related to the regulation of leaf width will be discussed.

*This study is supported by Monsanto Arabidopsis Microarray Program.

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45 KNAT1 regulates Arabidopsis shoot architecture

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Members of the knotted-like homeobox (knox) gene family encode putative transcription factors hypothesized to regulate shoot apical meristem development in flowering plants. Here we show that a loss-of-function mutation in the Arabidopsis knox gene KNAT1 underlies the brevipedicellus (bp) phenotype, characterized by short internodes and pedicels, turns at the nodes, and downward-oriented buds, flowers and siliques. Comparison of cell and internode lengths in wildtype and bp plants indicates that defects in both cell division and cell elongation contribute to the mutant phenotype. Inspection of bp internodes demonstrates that the mutant produces a stripe of tissue that winds around the stem and terminates on the abaxial surface of pedicels. Furthermore, epidermal cells of the stripe are reduced in size and lack stomata, while subepidermal layers contain larger cells with reduced intercellular spaces and fewer chloroplasts. Presence of the stripe over vascular bundles suggests that KNAT1 is required for tissue differentiation in a vasculature-dependent manner. Genetic studies indicate that KNAT1 interacts both additively and synergistically with the ERECTA gene to regulate shoot architecture. Our results implicate a role for knox genes in non-meristematic tissue as regulators of cell division, cell elongation and cell differentiation.

46 Cell fate determination in the shoot meristem

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The fate of stem cells in plant meristems is governed by directional signalling systems that are regulated by negative feedback. In Arabidopsis, the CLAVATA (CLV) genes encode the essential components of a negative, stem cell restricting pathway. Signalling through the CLV pathway limits the activity of the WUS gene, encoding a homeodomain transcription factor which promotes stem cell fate. This mutual regulation, involving positive and negative interactions, provides a feedback system for maintaining the delicate balance required for proliferation of stem cells to proceed at the right time and in the right place.

Using a transposon based activation tagging system, we have now identified additional genes that are involved in the control of stem cell fate in Arabidopsis. Mutations in one of these genes, *DORNRÖSCHEN*, affect the expression patterns of both WUS and CLV3 in the shoot meristem.

47 Cellulose biosynthesis and cell elongation

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Cellulose plays a central role in plant development. The orientation of microfibrils is regulated and controls growth anisotropy and cell shape. The acquisition of the ability to control the orientation of microfibrils appears to have been a crucial event in the colonisation of terrestrial ecosystems. Understanding cellulose synthesis and deposition is therefore essential for understanding plant growth, development and evolution. Based upon a screen for mutants with radially expanded hypocotyls combined with FTIR microspectroscopy, cellulose-deficient mutants have been isolated. Mutants in cellulose synthase isoforms (Fagard et al., 2000), a membrane-bound endo-1,4-ß-glucanase (EGase, Nicol et al., 1998) and a novel predicted integral membrane protein have been identified. In addition, we have shown that *ixr2*, a mutant resistant against the cellulose synthesis-inhibiting herbicide isoxaben, is mutated in the cellulose synthase family member CESA6. Knock-out mutants in the same gene show a short hypocotyl phenotype in dark-grown seedlings. This phenotype is conditional and is bypassed upon stimulation of phytochromes, suggesting that cellulose synthesis is under phytochrome control. The analysis of transcript levels for all ten cellulose synthase genes shows that this regulation is not at a transcriptional level. Mutations in *KOR*, encoding a membrane-bound EGase cause a deficiency in cellulose and do not affect xyloglucans, indicating that the enzyme is directly involved in the synthesis of microfibrils. We show that the enzyme is a part of high molecular weight complex that can be observed in Arabidopsis seedlings, but also in cotton fibres. Interestingly, the molecular weight of the complex changes during cotton fibre development, suggesting that KOR interacts with different partners at different growth stages. Potential roles for the enzyme in the synthesis of cellulose will be discussed.

Ref: Nicol et al 1998 EMBO J., 17, 5563-5576. Fagard, et al. 2000 The Plant Cell, 12, 2409-2423

48 A temperature-sensitive HEAT repeat identifies MOR1 as an essential microtubule associated protein

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Using a mutational strategy to identify microtubule organizing factors in *Arabidopsis thaliana*, we isolated two temperature-sensitive mutant alleles of the *MICROTUBULE ORGANIZATION 1* (*MOR1*) gene. This gene encodes a predicted 217 kDa protein that is the plant version of a recently discovered, ancient family of microtubule associated proteins, including human TOGp and *Xenopus* XMAP215 (Tournebize, R. et al. 2000, Nature Cell Bio. 2, 13-19). We found that both mutations occur within the same exon and that each substitutes a single amino acid residue within a conserved N-terminal HEAT repeat (Whittington et al 2001, Nature, in press). These N-terminal mutations impart reversible, temperature-dependent cortical microtubule disruption in interphase and terminally differentiating cells, leading to severe morphological defects. In contrast, mitotic and cytokinetic microtubule arrays remain intact in the *mor1-1* and *mor1-2* mutants and cell division patterns are not altered at the restrictive temperature. This suggests that MOR1 is essential for stabilizing cortical microtubules in expanding cells but not required for preprophase band, spindle or phragmoplast function. On the other hand, all MOR1 homologues associate with mitotic arrays so we considered the possibility of multiple copies. *MOR1*, however, occurs as a single copy gene in *A. thaliana* so cell cycle specificity may instead rely on transcriptional regulation. The N-terminal HEAT repeat targeted in our temperature-sensitive mutants could be a microtubule-binding site. Alternatively it could, like some HEAT repeats, confer plasma membrane localization. So far, mutant phenotype analysis supports either hypothesis.

49 Arabidopsis *GEMINI POLLEN1* is a putative microtubule-associated protein homologous to human ch-TOGp

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Pollen mitosis I (PMI) produces two unequal daughter cells, the vegetative and generative cells, that have different structures and developmental fates. The larger vegetative cell (VC) produces the pollen tube, whereas the smaller generative cell divides to produce two sperm cells. The *gemini pollen1* (*gem1*) mutation acts gametophytically to affect cytokinesis at PMI resulting in altered cell division symmetry and cell fate (Park et al., 1998, Development, 125: 3789). *gem1* produces a proportion of twin-celled or unequally-divided pollen, which adopt VC fate and express the VC-specific marker *lat52-gus*. Ultrastructural analysis revealed that cytokinesis is spatially uncoupled from nuclear division at PMI. Complete or partial ectopic internal walls with highly complex profiles divide the cytoplasm into twin or multiple cell compartments (Park and Twell, 2001, Plant Physiology, 126: in press). All cell compartments adopt VC fate with regard to lipid body distribution, an ultrastructural marker of VC fate. These observations suggest that altered cell fate in *gem1* results from abnormal inheritance of cell fate determinants as a result of disturbed cytokinesis. *gem1* was mapped to a 50kb region containing 9 putative genes on chromosome 2 (68cM). A single binary cosmid within this region was shown to complement *gem1*, resulting in a reduced frequency of aberrant, twin-celled pollen. This cosmid contains a complete copy of a putative Arabidopsis homolog of the human *ch-TOG* which is highly expressed in human tumors and brain. TOGp is a large (218KDa) microtubule and spindle-associated protein closely related to the MINI SPINDLES in drosophila. Mutation of *mini spindles* disrupts spindle assembly and mitotic chromosome segregation. Phenotypic analysis of *gem1* also suggests that GEM1 may function in spindle dynamics and/or cell plate guidance at PMI. The expression and potential role of the homologous GEM1 protein in microtubule dynamics will be discussed with regard to the effect of *gem1* on division symmetry and cytokinesis.

50 An Arabidopsis SNARE complex involved in Golgi-to-prevacuolar vesicle trafficking contains two syntaxins

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The syntaxin family of SNAREs (soluble N-ethyl maleimide sensitive factor adaptor protein receptors) is known to play an important role in the fusion of transport vesicles with specific organelles. Syntaxins form a t-SNARE complex with 2 other SNAREs on a target membrane. This complex then functions to recognize a v-SNARE embedded in the vesicle membrane creating a 4-helix *trans*-SNARE complex that drives fusion of the vesicle with the target membrane. Twenty-four syntaxins are encoded in the genome of the model plant *Arabidopsis thaliana*. These 24 genes are found in ten gene families and have been reclassified as SYPs (Syntaxins of Plants). Despite the presence of gene families, each syntaxin has a unique essential function as indicated by the lethality of gene disruptions in individual syntaxin genes. The SYP2-type syntaxins are found on the prevacuolar compartment (PVC). SYP4-type syntaxins are found at the *trans*-Golgi network (TGN), though the two most divergent members, SYP41 and SYP42, are found on distinct domains of the TGN. We have begun an investigation into the SYP5-type syntaxins, a group of syntaxins localized to multiple compartments of the endomembrane system including the TGN and the PVC. At the TGN, SYP5-type syntaxins are found predominantly on the SYP42-domain of the TGN, while they colocalize at the PVC with both SYP2-type syntaxins. On the PVC, a SYP2- and a SYP5-type syntaxin, together with the SNARE VTI11, form a novel two-syntaxin SNARE complex that is likely involved in TGN-to-PVC vesicle trafficking. Since a functional SNARE complexes require four SNAREs, ongoing research is aimed at identifying the fourth member of this complex, as well as other factors that interact with these syntaxins.

51 Characterization of the female gametophytic mutant *feronia* in *Arabidopsis thaliana*

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Pollination is a key step in the sexual reproduction of land plants. Sperm cells are delivered by a pollen tube - an extension of the male gametophyte - to the female gametophyte harboring the egg cell. In angiosperms, the pollen tube has to grow through the gyneocium to reach the female gametophyte - the embryo sac – and release the sperm cells for successful fertilization. How the pollen tube is guided to the embryo sac and what signals are involved in the correct timing and localization of the sperm cell delivery is poorly understood. In a screen of *Ds*-insertion lines of *Arabidopsis thaliana* for female gametophytic mutants we identified the *feronia* mutant that shows a defect in the release of sperm cells. Instead of bursting after reaching the embryo sac the pollen tube continues to grow and winds around the egg apparatus. The molecular and cytological characterization of the *feronia* mutant will provide new insights in molecular interaction between the male and female gametophyte and the processes controlling fertilization in flowering plants.

52 Regulation of floral homeotic genes by Polycomb-group genes in Arabidopsis

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The identity of floral organs is specified by homeotic genes, so that the particular combination of genes active in a whorl determines the type of organ that is formed. In several cases it has been shown that homeotic genes are required persistently during flower development for correct fate specification to occur. This raises the problem of how homeotic gene expression patterns that are set up early in development persist through cell division as the flower grows. We are studying the role of the Polycomb-group (Pc-G) genes, which in animals maintain patterns of homeotic gene expression by modifying chromatin structure so that on or off states of activity are inherited through somatic cell division. Previously we showed that the Arabidopsis CURLY LEAF (CLF) gene shows sequence homology with the *Drosophila* Pc-G member *Enhancer of zeste*, and that it has a similar role in maintaining repression of homeotic genes during leaf and flower development. Analysis of transgenic plants expressing a steroid inducible CLF construct suggests that the CLF protein is persistently during development in order to maintain silencing of a key target, the floral homeotic gene AGAMOUS. Although CLF RNA is present in all floral whorls, its action as a repressor of AGAMOUS is confined to whorls 1 and 2. Analysis of transgenic plants that express GFP-tagged CLF suggest that the CLF protein is also expressed in all floral whorls, and thus that its action as a repressor may occur by interaction with more localised factors. We therefore conducted genetic and molecular screens for proteins interacting with CLF and have identified two additional Pc-G members, *CURLY LEAF LIKE1/EZA1* and MOE LEAF . Characterisation of these genes will be presented.

53 Functional analysis of SUPERMAN gene in floral meristem

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The *SUPERMAN* (*SUP*) gene, which encodes a C2H2-type zinc-finger protein, is involved in cell proliferation of stamen and carpel primordia in Arabidopsis. *SUP* expression is initiated in the adaxial part of the stamen primordia in whorl 3 at stage 3 (early stage expression) and this expression is maintained until stage 8. Later at stage 9, *SUP* starts to express in the developing ovary in whorl 4, first on the inner surface of the carpels, and later in the funiculus of each ovule (late stage expression). As a first step of *SUP* gene functional analysis, we started a cis-element analysis of *SUP* gene. The *SUP* promoter has discrete cis-acting elements required for *SUP* expression in stamen primordia at early stages and in the ovary at later stages. Cis-elements required for expression in stamen primordia in whorl 3 confer strong gene expression in whorl 3 and whorl 4 from stage 3. This cis-activity at early stages is negatively controlled in mRNA regulation by two context-dependent cis-elements located within the coding region corresponding to the N-terminal zinc-finger domain. In contrast, cis-elements for ovary expression at late stages are not context dependent. A reporter gene construct that has GFP at the very end of the SUP coding region shows a nearly wild-type SUP expression pattern and also complements the *sup* mutant. Next, we generated a SUP inducible system by making a fusion protein with glucocorticoid receptor (GR) domain. In this system, the *sup* mutant was complemented in a DEX-dependent manner. By introducing tandem 35S enhancers in the upstream region of the *SUP* promoter, the SUP-GR fusion protein was ectopically induced. Under a DEX-induced condition, carpel numbers are increased and the inflorescence meristem terminates with carpelloid organs. Based on the SUP expression pattern and ectopic expression analysis in wild-type and several mutant backgrounds, a new model of SUP function will be presented.

54 Roles of *SEEDSTICIK* MADS-box gene during ovule and seed development

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One of the final steps of fruit development involves the formation of a seed attachment site at the juncture between the funiculus and the seed. Abscission within this site is required for the normal process of seed dispersal. The *SEEDSTICK* (*STK*) (formerly known as *AGL11*) MADS-box gene is required for the proper formation of this abscission zone since *stk* mutant seeds fail to detach from the funiculus. The *STK* gene also regulates growth of the funiculus, which becomes greatly enlarged in *stk* mutants during fruit development. This enlargement is caused both by changes in cell number and cell size. Mature seeds of *stk* mutants are also slightly smaller than those of the wild type and are abnormally shaped, suggesting a role of *STK* in integument development.

Because *STK* is closely related to the *SHATTERPROOF* (*SHP1* and *SHP2*) MADS-box genes, and all three genes share partly overlapping patterns of RNA accumulation in developing ovules, we constructed the *stk shp1 shp2* triple mutant to investigate possible genetic redundancy. Strikingly, normal ovule development was completely disrupted in the triple mutant and the mutant ovules were converted into carpel-like structures. These results suggest that the *STK* and *SHP* genes share overlapping activities that are required for ovule identity. *FRUITFULL* (*FUL*), a MADS-box gene involved in carpel valve cell differentiation, is ectopically expressed in the converted ovules suggesting that *STK*, *SHP1* and *SHP2* are redundant negative regulators of *FUL* in ovule. Whether or not ectopic expression of *FUL* is necessary for the conversion of ovules toward carpels is currently being investigated. Genetic interactions between *STK* and other genes, including *AINTEGUMENTA* (*ANT*), *INNER NO OUTER* (*INO*), *BELL1* (*BEL1*) and *APETALA2* (*AP2*) will be presented.

55 Molecular and genetic approaches to identify regulators of *AGAMOUS*

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Traditional mutant analyses have not identified region-specific activators of the floral homeotic gene *AGAMOUS (AG)*, which specifies stamen and carpel fate in the center of flowers. This could either be due to redundant regulation of *AG*, or to the fact that important *AG* activators are essential at earlier developmental stages. To circumvent these problems, we are using two complementary strategies to elucidate the molecular mechanisms underlying *AG* regulation.

(1) Enhancer mutagenesis. We have performed a screen for mutations enhancing the phenotype of *wonderwoman-1 (wow)* mutants (a gift from Y. Eshed and J. Bowman, UC Davis), which show dosage-sensitive interactions with *ag* (Y. Eshed & J. Bowman, pers. communication). We have isolated several enhancer mutations that together with *wow* show various degrees of stamen-to-petal transformation. Mapping indicates that they correspond to new loci.

(2) Dissection of the *AG* enhancer. We have identified several candidate transcription factor binding sites in the *AG* enhancer by comparison with consensus binding sites for known classes of transcription factors. Some of these are conserved in other species, as revealed by sequencing over 20 *AG* genes from other members of the Brassicaceae (see poster by Hong et al.). We have identified one factor that is expressed in flowers and that can bind two of the conserved sites in vitro. The significance of the binding sites in planta is being studied by mutating them in the context of *AG::GUS* reporters.

This work has been supported by postdoctoral fellowships from the Studienstiftung des Deutschen Volkes and BASF (J.U.L.) and Human Frontiers Science Program Organization (J.U.L.), NIH training grant (R.H.), and by a grant from DOE (D.W.).

56 A Mitochondrial Complex I Defect Impairs Cold Regulated Nuclear Gene Expression

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Low temperature is an important environmental factor influencing plant growth, development, and geological distribution. Due to their sessile nature, plants have developed unique mechanisms to cope with cold stress. In order to elucidate low temperature signal transduction in plants, we have previously developed stress-inducible bioluminescent *Arabidopsis* plants that express firefly luciferase driven by the stress responsive *RD29A* promoter. Using this system, mutants defective in stress responses were identified based on their abnormal luminescence under different stress conditions. Here we report on the characterization and cloning of one mutant, *frostbite1* (*fro1*), which shows reduced luminescence induction specifically by cold stress. *fro1* mutant plants display lower levels of cold induction of stress responsive genes such as *CBF2*, *RD29A*, *KIN1*, *COR15a*, and *COR47*. *fro1* mutant leaves appear water-soaked and resemble wild-type leaves that have been subjected to freezing stress. Electrolyte leakage tests showed that *fro1* mutant leaves are constitutively leaky and have reduced capacity for cold acclimation. The *FRO1* gene was isolated through map-based cloning. *FRO1* encodes a protein with high similarity to the 18 kD Fe-S subunit of Complex I (NADH dehydrogenase, EC 1.6.5.3) in the mitochondrial electron transfer chain. Confocal imaging showed that the FRO1:GFP fusion protein is localized in mitochondria. The Complex I defect in *fro1* results in constitutive accumulation of reactive oxygen species that may modulate calcium signaling and cold-responsive nuclear gene expression. These results illustrate that nuclear gene expression under low temperature conditions is coupled with mitochondrial function.

57 The *Arabidopsis HOS1* gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleo-cytoplasmic partitioning

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Low temperature is one of the most important environmental stimuli that control gene transcription programs and development in plants. In *Arabidopsis thaliana*, the *HOS1* locus is a key negative regulator of low temperature-responsive gene transcription. The recessive *hos1* mutation causes enhanced induction of the CBF transcription factors by low temperature as well as of their downstream cold-responsive genes. The *hos1* mutant plants flower early, and this correlates with a low level of *Flowering Locus C* gene expression. The *HOS1* gene was isolated through positional cloning. *HOS1* encodes a novel protein with a RING finger motif near the amino terminus. *HOS1* is ubiquitously expressed in all plant tissues. HOS1-GFP translational fusion studies reveal that HOS1 protein resides in the cytoplasm at normal growth temperatures. However, in response to low temperature treatments, HOS1 accumulates in the nucleus. Experiments are underway to understand the significance of the RING finger motif in HOS1 protein.

58 Arabidopsis encyclopedia using full-length cDNAs and its application for expression profiling under abiotic stress conditions

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Full-length cDNAs are essential for functional analysis of plant genes[2)]. Using the biotinylated CAP trapper method, we constructed full-length cDNA libraries from Arabidopsis plants[1),2)]. Until now, we obtained 102,601 3'-ESTs. The 3'-end ESTs could be clustered into more than ca. 11,000 non-redundant groups. We are planning to collect more than ca. 15,000 independent cDNA groups in the future. We determined full-length cDNA sequences of ca. 1000 RAFL cDNA clones in collaboration with the Arabidopsis SSP group of the USA(PI: Drs. Ecker, Theologis and Davis) as of April 18, 2001. We are planning to determine full-length cDNA sequences of ca. 10,000 RAFL cDNA clones. After determination of full-length cDNA sequences, the RAFL cDNA clones will be distributed from the RIKEN Bioresources Center and ABRC. We have used the full-length cDNAs for the microarray analysis of expression profiles of Arabidopsis genes under drought, cold and high-salinity-stresses[3)]. Our previous results showed that the full-length cDNA microarray is a useful material to analyze the expression pattern of Arabidopsis genes under drought and cold stresses, and to identify target genes of stress-related transcription factors and potential cis-acting DNA elements by combining the expression data with the genomic sequence data[3)]. Recently, we prepared a new version of full-length cDNA microarray containing ca. 7000 independent full-length cDNA groups to analyze the time course of gene expression in response to drought-, cold-, high salinity- and ABA-treatments. In this meeting, detailed characterization of the drought-, cold-, high-salinity- and ABA-inducible genes will be presented. 1) Seki et al. (1998) Plant J. 15: 707-720. 2) Seki et al. (2001) Plant Physiol. Biochem. 39: 211-220. 3) Seki et al. (2001) Plant Cell 13: 61-72.

59 The identification of transcription factors regulating temperature and water stress responses

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Plant growth is strongly inhibited by abiotic stresses such as low temperature and drought. In response to those stresses, plants have evolved adaptive mechanisms which include the induction of a variety of genes (for example, the cor, rd, erd and lti genes). So far about 80 water stress responsive genes have been reported. However, because the biochemical and physiological changes during stress adaptation are complex, this number will probably increase. The coordinated induction of such a large number of stress responsive genes are likely controlled by regulatory proteins such as transcription factors. The completion of the Arabidopsis genome sequencing project for the first time provides the opportunity to identify all transcription factors involved in the stress response through functional genomics on a genome wide scale. We are using a reverse genetics approach, i.e. a combined approach of gene overexpression and knock-outs, to understand the roles transcription factors play during adaptation to various stresses. I will show results from the analysis of the first 500 transcription factors from Arabidopsis, and specifically, results that are related to temperature and water stress responses. As an example, I will focus on the discovery of a new transcription factor that is part of the water stress response pathway in Arabidopsis, and illustrate how this discovery is providing new insights in the understanding of stress adaptation by plants.

60 A mRNA cap binding protein, ABCAP, modulates early abscisic acid signal transduction in Arabidopsis.

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Abscisic acid (ABA) is a plant hormone that regulates important stress and developmental responses. A new recessive ABA hypersensitive mutant, *abcap*, was isolated. ABCAP encodes a nuclear mRNA cap binding protein homolog that together with a newly identified cap binding complex subunit, AtCBP20, binds mRNA cap structure. Analyses of responses to other plant hormones show ABA specificity. DNA chip and northern analyses show that only a few transcripts are down-regulated in abcap several of which are implicated in ABA signaling. *abcap* plants show ABA-hypersensitive stomatal closing, reduced wilting during drought and, interestingly, ABA-hypersensitive cytosolic calcium increases in guard cells, demonstrating amplification of early ABA signaling mechanisms. ABCAP represents a novel mechanism for modulation of ABA signaling by a proposed transcript alteration of early ABA signaling elements.

61 Effects of ectopic *SUPERMAN* and *CRABS CLAW* expression on regulation of *INNER NO OUTER* and ovule morphogenesis

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The *Arabidopsis* ovule can serve as an useful model for polar growth and development in plants. The expression pattern of a key regulator of ovule development, *INNER NO OUTER* (*INO*), not only responds to the proximal/distal axis of the developing primordia but may also influence the abaxial/adaxial domains of the emerging outer integument. Maintenance of the normal expression pattern of *INO* within the ovule primordia requires *SUPERMAN* (*SUP* or *FLO10*), a known regulator of both floral and ovule development. Using the INO promoter to express *SUP* inhibits the growth of the outer integument and provides additional evidence that SUP is formally a negative regulator of *INO*. In contrast, expression of *CRABS CLAW* (*CRC*), a gene closely related to *INO*, under the INO promoter increased growth of the adaxial domain of the outer integument and indicates that the *INO* coding region or protein may be directly involved in regulation by SUP. Analysis of reporter gene expression from the INO promoter in several mutant and transgenic backgrounds is being used to further clarify the regulatory interactions of these genes.

62 Non-cell autonomous function of SHORT-ROOT in root radial pattern formation: 2. Effect of ectopic SHR expression

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Radial pattern formation in the *Arabidopsis* root is a result of stereotypical cell divisions and acquisition of correct cell fates. Previous studies have suggested that this pattern formation relies on transmission of positional information between root cells by as yet uncharacterized mechanisms. The *SHORT-ROOT (SHR)* gene has been implicated in positional signaling from the stele to the adjacent cell layer, because the gene is expressed exclusively in the stele but affects the cell fate of the surrounding endodermis. Moreover, *SHR* is necessary to maintain the expression of *SCARECROW (SCR)*, which is expressed in the layer adjacent to the stele. In order to understand the role of SHR in the adjacent layer, *SHR* was directly expressed in this layer using the *SCR* promoter. The resulting transgenic plants acquired a novel radial pattern phenotype in the root. Analysis of cell type specific markers in these transgenic plants revealed a relatively simple mechanism that controls both cell division and differentiation in a position-dependent manner.

63 A gain-of-function mutation at the *TOPLESS* locus causes shoot to root transformations during embryogenesis

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The temperature sensitive *topless-1* (*tpl-1*) mutation transforms the shoot apical meristem (SAM), the cotyledons, and hypocotyl into a second root during embryogenesis. Analysis of markers for the SAM and cotyledons (*SHOOT MERISTEMLESS* and *AINTEGUMENTA*) show that the expression of these genes is either absent or reduced, respectively. Conversely, the expression of *SCARECROW*, which marks an endodermal layer in the hypocotyl and root of wild-type embryos, is expanded into the apical half of *tpl-1* embryos as early as the globular stage. Expression of a root specific GUS reporter, LENNY, is found at both poles in *tpl-1* and an auxin responsive GUS reporter, DR5, also shows misexpression in *tpl-1* embryos. We have cloned *TOPLESS* using a map-based approach and show that it encodes a large protein containing several predicted WD40 repeats. *TPL* belongs to a 9 member gene family in Arabidopsis. Genetic data indicate that *tpl-1* is a gain-of-function mutation and intragenic suppressors have been isolated.

64 A stereotyped mode of death in Arabidopsis cells abutting wound sites.

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We have used a series of targeted GFP markers to create kinematic portraits of the subcellular events that occur in response to wounding using live-cell time-lapsed confocal microscopy. These studies reveal that a subset of cells directly abutting wound sites undergo a stereotyped mode of cell death that occurs 1 - 2 hours after wounding. Numerous events occur during this time including nuclear contraction, nuclear lobing, release of nuclear contents, disintegration of the cortical ER network and ultimately cellular collapse and cell death. The simultaneous contraction and lobing of nuclei is shown to involve the separation of the membranes of the nuclear envelope. We also describe rapid wound-induced alterations in the structure of fusiform bodies, ER accessory bodies of unknown function, providing direct evidence that these organelles are structurally dynamic and responsive to wounding.

To probe the specificity of these events we have characterized chemically induced cell death using the herbicide chloroxynil which triggers many of the events induced by wounding such as callose deposition, fusiform body alterations and cellular collapse, but not nuclear contraction. Thus, nuclear contractions can be separated from cellular collapse and other events, suggesting there is specificity to this aspect of wound-induced cell death.

Collectively, our observations suggest a previously unrealized level of complexity in local subcellular responses to wounding and demonstrate similarities between wounding and the hypersensitive response to pathogen attack at the cellular level. Our observations raise mechanistic questions as to how wound-induced cellular responses are executed, whether they are controlled by the same regulatory machinery that controls the dynamics of the hypersensitive cell death response, and ultimately, what their underlying functions are. As one explanation, we propose that cell death may function to limit pathogen exploitation of cells compromised by wounding.

65 Analysis of Photoprotection-Deficient *Arabidopsis* Mutants Acclimated to High Light

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Plants grown in low light (LL) often suffer photoinhibition and oxidative damage when exposed to high light (HL). In contrast, plants that are grown in HL are able to acclimate to this environment and do not show the symptoms of light stress. Rapidly induced mechanisms, such as thermal dissipation of excess absorbed light (qE), are thought to protect plants during short-term light stress. *Arabidopsis* mutants that are deficient in qE and/or the synthesis of specific carotenoids are able to acclimate to HL, suggesting that these mechanisms are not essential for long term photoprotection. To uncover photoprotective mechanisms that can compensate for the lack of qE and carotenoids, we analyzed HL and LL wild-type plants and mutants of the following genotypes: (1) *npq4*, which lacks qE, (2) *npq4npq1* double mutant, which also fails to accumulate the carotenoid zeaxanthin when exposed to HL, and (3) *npq4npq1lut2* triple mutant, which also fails to accumulate the carotenoid lutein. Leaf pigment analysis showed that all plants grown in HL had a bigger total carotenoid pool size and higher levels of tocopherols. All mutants had significantly higher levels of tocopherols in HL than the wild type, suggesting a possible compensation for the photoprotection deficiencies. Photosynthetic electron transport was similar between mutants and wild type in both HL and LL conditions. All HL plants had thicker leaves, due to both additional cell layers and larger mesophyll cell size, and the ratio of stomates to epidermal cells was also higher. At the chloroplast level, all HL plants showed much less thylakoid stacking and starch granule size and number than LL grown plants. In the mutant plants, but not in wild type, we observed chloroplast vesicles of different sizes. In LL plants, these vesicles occurred at a very low frequency, whereas in HL plants we saw chloroplast vesicles at a very high frequency, particularly in the double and triple mutants. The contents of these vesicles appeared to be cytoplasmic in origin. Our results suggest that deficiencies in qE and/or specific carotenoids can affect chloroplast morphology in HL, but can be compensated for by alternative photoprotective mechanisms, such as elevated levels of antioxidants.

66 Identification of herbicide targets using a high-throughput functional genomics approach

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Industrialized plant functional genomics can quickly lead to the discovery of novel herbicides and the simultaneous development of crops resistant to these new herbicides. Herbicides function through an interaction with essential proteins (considered herbicide targets). A useful herbicide target is one that is required for viability but is distinct from proteins in non-pest organisms. One way to discover new targets is through using a combination of comparative and functional genomics. Gene sequences can be compared within a genome and across genomes to identify plant genes that are likely to be essential yet distinct from animal genes. This comparative work can be complemented by a more direct experimental approach in which essential genes would be identified by virtue of a lethal phenotype when “knocked out.” We have used an approach by which pre-determined genes are “knocked out” by antisense or gene silencing in conjunction with a transactivation system. In such a system, an antisense construct corresponding to a putative target gene is present in a latent state until combined with a transgene designed to drive expression of the target transgene. The transgenes can then be combined by sexual crossing and the resulting F1 population screened. This strategy provides an added advantage in that one is able to immediately know the identity of the target gene, unlike with chemical or insertional mutagenesis. A validated target gene could then be expressed and the protein screened in vitro against diverse chemical libraries of compounds in a high-throughput platform. Any hits resulting from such an assay would then lead to in vivo microscreening. Synthesis of the lead compound followed by greenhouse and field-testing would be the final steps in product development. We will describe our high-throughput screening procedure and describe examples of our results.

67 The analysis of the effects of RNA type and labeling on microarray results

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There are a number of different methods being used in probe preparation for microarray analysis. We used different techniques to label RNA to determine variations within these methods. One consideration is the use of total RNA or polyA+ extracted RNA. These can be labeled either by 1-step labeling, where fluorescent dye is directly incorporated into the reverse transcription, or 2-step, where a first strand cDNA is generated then fluorescent dye is incorporated in a random primed reaction using Klenow. The Arabidopsis Functional Genomic Consortium has constructed a microarray containing about 11,000 non-redundant ESTs, which is available for the use of the Arabidopsis community. Using this array, two of these parameters were tested. First, total and polyA+ RNA labeled with the 1-step method, and second, polyA+ RNA labeled with 1-step and 2-step method were compared. The ratios between the hybridizations were analyzed. Using stringent spot quality selection criteria, approximately 6,500 ESTs from 4 repetitions were analyzed. Of these, 2-2.5% showed a greater than 2-fold ratio, suggesting that careful consideration is needed when choosing a labeling strategy.

68 The German Plant Genome Research Initiative (GABI)

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The Genome Analysis in the Biological System Plant-GABI is a joint research program supported by the German Ministry of Education and Research (BMBF) and the German industry. GABI was started in the late 1999 and is being funded for a period of four years. Out of 190 submitted pre-proposals an international panel of reviewers recommended 71 projects with a total financial volume of more than 80 million DM for further support. Two main research areas are bunching these activities. The Research Area I covers fundamental research activities, while the Research Area II comprises more applied activities. Research is being conducted on eight different plant species (Arabidopsis, barley, sugar beet, rape-seed, potato, poplar, maize, and rye) focussing on the model species Arabidopsis and barley. Nearly 44% of the total financial support are being spent for the research in these two model species to support 27 single projects. Three resource centres are developing and providing access to technologies and are generating resources. Furthermore, two bioinformatics resource centres are providing platforms for data handling and storage, and developing tools for their analysis and display. 30% of the financial resources in GABI help the activities of the resource centres. The remaining 26% are being spent in order to support the research in the six other species with a special focus on sugar beet. A patent and licence agency was founded in GABI in order to commercialise and protect the intellectual property rights.

69 The FST project: ESTABLISHMENT OF BIOLOGICAL AND COMPUTER RESOURCES FOR ARABIDOPSIS FUNCTIONAL GENOMICS

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The aim of the project is to set up biological and computer resources allowing to obtain mutants for genes of known sequences, in order to study their function (*in silico* reverse genetics). The biological resource is a collection of T-DNA insertion lines of *Arabidopsis thaliana* (50 000 primary transformants) from the "Station de génétique" (INRA, Versailles). The plant material is produced from theses lines for the molecular characterisation of the insertion sites of the T-DNA ("FST" for Flanking Sequence Tag). After genomic DNA extraction, FSTs are PCR amplified and sequenced (Balzergue *et al*., 2001). The sequences are then processed and compared to databases. FSTs are located on the Arabidopsis genome and graphical outputs show relative positions of predicted genes as well as FSTs among BACs. All the data (FSTs, molecular data) are stored and managed through an Oracle relationnal database (FLAG-db). Requests are made through a web server using a blast interface allowing to position any nucleotide sequence on the genome and then localise the FSTs in the vicinity or within the target sequence. Altogether, this constitutes a powerful tool for functional genomics in plants. Our data suggest that a fully exploitable FST is obtained for 60% of the insertion lines processed. This includes lack of primary amplification (16%), bad sequencing (16%) and tandem inserts (8%). To date 6.000 FST have been obtained. A public database regularly updated (10.000 / year) should be opened in June 2001.

Balzergue *et al.* (2001) Biotechniques, 30, 496-504.

70 Cis-Element/Transcription Factor Analysis (cis/TF): A Method for Discovering Transcription Factor cis-Element Relationships

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In an effort to use computational techniques to reveal function in the Arabidopsis genome, we report a simple new algorithm, cis/TF, that uses genome-wide expression data and the full genomic sequence to match transcription factors to their binding sites. Most previous computational methods discovered binding sites by clustering genes having similar expression patterns and then identifying over-represented subsequences in the promoter regions of those genes. cis/TF asserts that b is a likely binding site of a transcription factor T if the expression pattern of T is correlated with a measure of the composite expression patterns of all genes containing b, even when those genes are not mutually correlated. Thus, our method focuses on binding sites rather than genes. In a test, the algorithm has successfully identified experimentally-supported transcription factor-binding relationships in tests with independent datasets from Saccharomyces cerevisiae. Our goal is to apply this computational method to cell-type specific expression profiles we are generating in Arabidopsis.

71 Enhancer-trap lines for targeted misexpression

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As part of a conditional activation tagging project, we have generated a collection of 5000 enhancer trap lines utilising the pBIN mGAL-mGFP5 HDEL #15 construct, produced and tested by Dr J. Haseloff (University of Cambridge, UK). The construct is currently being modified to include a GUS reporter gene (additional to the GFP5 already present) to facilitate visualisation of weak expression patterns. At the time of writing more than 2,000 transformants have been characterized and approximately 200 lines expressing GFP at the seedling stage have been identified. In the majority of these lines GFP expression appears to be tissue, or organ specific. Further screening and more extensive characterisation of GFP expression will also be carried out in mature plants and embryos.A second collection of transgenic plants is being established by random insertion of UAS response elements in the background of an inducible GAL4 source. Activation-tagging insertions preselected for conditional dominant phenotypes can be crossed into the above enhancer-trap lines to restrict expression to specific spatial and temporal patterns. We are particularly interested in genes influencing embryo and vascular pattern formation.

72 Transcription Profiling of the *Arabidopsis* transcription factors in response to environmental stresses and developmental cues.

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Plants have developed various mechanisms to respond to different environmental and developmental stimuli. One type of responses is through activation or repression of gene expression. Plant transcription factors, as one of the final components in the signal transduction pathways, play an important role in governing gene expression. In order to understand the molecular mechanism by which plant transcription factors regulate plant development and responses to genetic and environmental changes, we have studied more than 400 transcription factors, including those belong to the AP2/EREBP family, the AtMyb family, the bZIP family, and the zinc finger transcription factors, some of which are involved in plant stress responses, and monitored their behavior in response to developmental cues using the high-density oligonucleotide probe array (GeneChip)[1]. Two families of *Arabidopsis* transcription factors, AP2/EREBP and WRKY, were closely monitored in major organs at different developmental stages, and in more than 80 different stress conditions, including biotic and abiotic stresses. Differentially expressed genes encoded transcription factors in roots, leaves, and flowers at different developmental stages, and in different stress conditions, were identified. Functional classification of genes, including those that encoding for transcription factors, based on expression pattern was determined. In addition, we have also investigated the gene expression profiles for these transcription factors in a number of mutant backgrounds and identified genes that are potentially the key regulators in plant defense responses. Our results provided insights into transcriptional regulation and coordination of gene expression in response to environmental and developmental stimuli.

1. Tong Zhu, et al. (2001) *Plant Physiol. Biochem.* 39: 1-22

73 Functional Genomics of Ozone Stress in *Arabidopsis*

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The molecular basis of ozone's (O_3) phytotoxicity is still not well understood. Recent evidence points to a relationship between O_3 stress and components of the well-characterized pathogen defense response in plants. Exposure of plants to high levels of O_3 leads to rapid formation of necrotic lesions and cell death, mimicking the hypersensitive response to pathogen infection. We have constructed a microarray containing more than 1,300 stress-specific elements prepared from *Arabidopsis* ESTs. These ESTs were obtained through subtractive hybridization experiments including O_3 exposure, salicylic acid treatment, and infection with bacterial and fungal pathogens. More than 500 of the genes corresponding to the stress-associated ESTs show a peak induction or repression of at least two fold when exposed to 350 ppb O_3 for 6 hrs. Of these, approximately 130 are induced within 1.5 hrs of the treatment. These include a wide range of characterized and putative transcription factors and signaling genes. Sequence analysis of the upstream regions of these "early response" genes will be conducted to look for common regulatory elements. These genes are also being targeted for RNAi knockout and overexpression to identify downstream stress response genes under their control or influenced by their expression. These will be identified by microarray expression analyses of mutant and wildtype plants under O_3 stress.

74 ZIGIA - A CENTER FOR FUNCTIONAL GENOMICS IN ARABIDOPSIS

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ZIGIA uses a collection of *A. thaliana* Columbia lines mutagenised with the autonomous maize transposon En-1/Spm (hereafter referred to as En) that currently consists of 11,000 individuals, each carrying 1-20 copies of the transposon, representing on average six independent insertions per plant. This population has been used successfully by groups from all over the world to study gene-function relationships using forward genetics (11 published mutants) as well as reverse genetic approaches (6 published mutants). Until September 2000 the population was screened by PCR on DNA pools. Last year we introduced a new method using DNA arrays, that allows a higher throughput. All transposon-flanking regions from individual plants are amplified by PCR and subsequently spotted at high density onto nylon membranes. A single hybridization experiment with a gene-specific probe then allows one to identify candidate mutant plants. Since the cloning of mutants discovered in the forward screens is frequently hampered by the occurrence of non-tagged mutation sites such as rearrangements (T-DNA lines) or footprints (En lines), we are also working on the optimization of the speed and resolution of the segregation analysis. We therefore introduced for Transposon Insertion Display a denaturing HPLC system, as an alternative to separation on agarose and acrylamide. By direct coupling the DHPLC to a microfraction collector, purified fractions are obtained that can be used, after reamplification, for sequencing. In addition the system is used for Transposon Footprint Analysis, e.g. to detect small polymorphism's in those cases where the presence of a non-tagged mutation is suspected. Here, ZIGIA presents recent experiences with these methods, including an estimation of their efficiency. Our screening facility can be accessed through the ZIGIA project at the Max-Planck-Institute for Plant Breeding Research in Cologne (http://www.mpiz-koeln.mpg.de/~zigia).

75 Transcriptional responses triggered by the *RPP7* defense signaling pathway

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Interactions between isolates of the Oomycete pathogen *Peronospora parasitica* (Peronospora) and distinct Arabidopsis ecotypes have been extensively used to reveal signaling pathways regulating plant disease resistance. Putative plant receptor proteins encoded by *RPP* genes (recognition of *P. parasitica*) mediate specific recognition of Peronospora isolates and trigger defense reactions. Two members of this class, RPP7 and RPP8, act via unconventional signaling cascades and are not dependent on previously established defense regulators, such as EDS1, NDR1, PAD4, NPR1, RAR1, PBS3 or salicylic acid. Three components of the *RPP7* pathway, EDM1, -2 and -3, have been identified by mutant screens. To define gene expression signatures specific for the *RPP7/8* pathways and to identify genes that may be crucial for effective Peronospora defense, we are currently performing large scale gene expression profiling using oligonucleotide chips representing roughly 8200 different Arabidopsis genes. Among genes showing Peronospora-induced expression changes clusters of genes specifically up-regulated by either RPP7 or RPP8 or both could be defined and categorized by their temporal behaviour. The promoters of early and transiently RPP7/8 activated genes were found to be significantly enriched with both novel sequence motifs and potential binding sites of known transcription factors. Comparisons between expression signatures of the *rpp7*, *edm1*, *-2* and *-3* mutants allow predictions of the *RPP7* pathway hierarchy.

76 Establishing a Gene Expression Atlas of Arabidopsis thaliana Using Integrated Genomic Approaches for Transcript Profiling

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Transcript profiling has transformed studies of gene expression so that mRNA levels of nearly all genes in an organism can be analyzed simultaneously. For any sequenced genome, such as Arabidopsis thaliana, transcript profiling can also ascribe roles to genes of unknown function or to genes identified solely by computational means. At Ceres, we have generated cDNA microarrays containing more than 11,000 cDNA clones, a majority of which are full-length clones. To ensure the quality of our microarrays, we have built a Laboratory Information Management System (LIMS) that tracks E. coli clones, plasmid DNA, PCR fragments, microarray features, experimental samples to data analysis. In collaboration with Keygene N.V. (The Netherlands), we have also established a comprehensive expression database for the majority of Arabidopsis genes using cDNA-AFLP™ technology, including those that produce low transcript levels. In this database, the cDNA-AFLP fragments are linked to their corresponding mRNAs so that information about the expression level of Arabidopsis genes can be obtained simply by querying the database with a cDNA-AFLP™ gel profile. In conjunction with GeneChips (Affymetrix), these technologies have provided us with coverage of more than 20,000 Arabidopsis genes, as much as 80% of the transcriptome. Using Ceres' annotation of the genome, we are also generating an even more comprehensive microarray-based view of the Arabidopsis transcriptome. We have analyzed a diverse range of samples representing various tissues/organs, developmental stages, environmental conditions, and a variety of mutants in order to establish an atlas of gene expression for Arabidopsis. By integration of information from other resources such as bioinformatics, genetic studies, and protein structures, etc, we are increasing our understanding of the function of Arabidopsis genes in the context of the whole genome and identifying candidate genes of agronomic importance.

77 Normalization, additive error and systematic bias in gene expression data

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Gene expression profiling techniques have inherent systematic biases that require detection and correction by normalization. Most normalization methods apply a function or constant to correct intensity. The iterative regression method calculates a linear function of intensity and reduces the variability of expression measurements, when compared to means methods. The linear model is based on a biological model of gene expression that assumes that for any given stimulus (y) the typical transcript level will not change and that atypical responsive transcripts can be identified by comparison to a control (x). Also assumed is that the relative abundance of a transcript does not influence the likelihood of that gene responding to a given stimulus. By this model, transcript levels should be fit the line $y = mx + b$ where the slope (m) is 1 and the y intercept (b) is zero. Real data is scaled to remove additive error, log transformed and regressed to determine m and b. Real normalization functions will differ from the ideal model due to both biological and technical causes. The temporary removal of outlying data points reduces the influence of biological responses on the normalization function. Iteration of the method insures robustness and improves accuracy. The applicability of the linear model is then tested with lack of fit methods. Nonlinear exceptions to the linear biological model are presumed due to technical causes. In these cases, a nonlinear model is required. Even the ideal fit of a intensity based normalization function cannot fully correct for some systematic biases. Examples of systematic spatial biases in cDNA microarrays are presented.

78 Genome Analysis of the SCF and HECT E3 Families in Arabidopsis thaliana

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Genetic analyses indicate that the ubiquitin/26S proteasome pathway is required for many aspects of Arabidopsis growth and development, including flower, leaf and embryo development, auxin and jasmonate responses, male gametogenesis, and circadian rhythms. The specificity of the pathway is largely determined by the ubiquitin ligases (E3s). They recognize appropriate targets and then facilitate the transfer of activated ubiquitin from E2s, resulting in the formation of an isopeptide bond using the C-terminus of ubiquitin and available lysines within the target. Currently there are four classes of E3s that have been identified in Arabidopsis: the HECT, SCF, Ring/U-box and APC E3s. The HECT E3 family shares a common 350-aa HECT domain at their C-terminus, which contains an positionally conserved cysteine essential for ubiquitin transfer. The N-terminal domain of these proteins is believed to be involved in the recognition of multiple substrates. Through genome analysis we predict that there are 9 HECT E3s in Arabidopsis varying in size from 100 to 405kDa. The SCF E3s are four subunit complexes whose specificity is determined by the F-box family of proteins. F-box proteins contain a ~60-aa N-terminal motif, the F-box, which allows them to associate with the rest of the SCF E3 complex. They also have a variable C-terminal domain that is responsible for target recognition. Analysis of the Arabidopsis genome identified >564 possible F-box proteins, making this class the most diverse component of the pathway. These proteins fall into multiple families based on their F-box motifs and on the nature of their protein-protein interaction domains. These domains include WD-40s, LRRs, Kelch and Armadillo-like repeats. We currently have identified T-DNA insertion mutants in 2 of the HECT E3s and 7 of the F-box E3 genes and are analyzing them phenotypically to determine their function(s) in Arabidopsis. Work supported by a USDA-NRICGP grant 00-35301-9040 to RDV.

79 Microarray Gene Expression Information in TAIR

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The Arabidopsis Information Resource (TAIR) (www.arabidopsis.org) is a comprehensive repository and information retrieval system for *Arabidopsis* genomic data. The first release of TAIR database included information about genes, markers, clones, maps, and community. The database will be expanded sequentially to accomodate other types of data, such as gene expression, publications, proteins, germplasm, and other relevant data extracted from the literature. One of our goals for the current year is to add *Arabidopsis* microarray gene expression data. Towards that end, we are developing a database structure that will hold both cDNA- and oligonucleotide-based array data, and eventually other types of expression data, such as in situ hybridization, northern, reporter genes, and RT-PCR. Our database model follows MGED (Microarray Gene Expression Database) Group standards to facilitate, among other things, comparability of gene expression data from different sources. Some of the functionalities of the microarray-expanded TAIR database will include import and export of expression data, searching expression patterns of genes by name or sequence, searching expression patterns by experimental conditions or biological samples, linking search results to TAIR gene detail pages and array elements to the TAIR Sequence Viewer, allowing access to pre-clustered data, and linking experiments to publications. In addition, we are putting a substantial effort into developing controlled vocabularies to describe TAIR data objects. We have joined the Gene Ontology consortium and are assisting in developing the GO to accomodate plant genes. We are also working to develop ontologies to describe treatments and biological samples (anatomy, developmental stages, and phenotypes), which will be crucial for describing not only microarray experiments, but also many other types of data in TAIR.

80 From microarray expression profiles to gene suppression and back.

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In our laboratory, we have constructed a cDNA microarray comprising ESTs for genes that are induced and repressed by biotic and abiotic stresses. These ESTs were obtained from PCR-suppression subtractive hybridization libraries prepared from *Arabidopsis thaliana* (Columbia ecotype) plants infected with either bacterial or fungal pathogens, exposed to ozone, or treated with either salicylic acid or methyl jasmonate. Expression profiles were obtained using cDNA from RNA isolated from plants exposed to different stresses as a probe. These microarray expression analyses have allowed us to identify specific sets of genes that are up- or down-regulated as consequence of a particular stress. We are now focussing on the functional and molecular characterization of some of those plant stress responsive genes with the intention of dissecting their role in the stress-signaling pathway. Our initial efforts have been directed at two transcription factors [*] and one mitogen activated protein kinase[*] that are induced early in plant stress responses. To suppress their expression, we have made traditional antisense and novel dsRNA constructs for these genes and introduced them in plants. Additionally, we have made and introduced over-expression constructs to increase the level of gene expression. We are beginning microarray expression analysis of the transgenic plants to identify downstream response genes controlled directly or indirectly affected by the targeted genes.

[*]GenBank Accession Numbers AAF16756, AB013887 and ATHATMPK3.

81 Identification of genes involved in seed quality in *Arabidopsis thaliana*

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As part of a Génoplante project (French Genomic Initiative), we are using the collection of *Arabidopsis thaliana* T-DNA insertion lines of Versailles (Genetics laboratory, INRA Versailles) to isolate mutants affected in seed maturation and quality. 20,000 T3 progenies have been visually screened for a 'wrinkled/shrunken' seed phenotype. Eighty lines have been selected for further study. Genetic (determinism and tagging of the mutation), biochemical (carbohydrates and lipids) and molecular analyses (number of T-DNA, cloning of the Flanking Sequence Tag (FST) and expression) are carried out on seeds propagated under controlled conditions.

As a reference, study of carbohydrate (glucose, fructose, sucrose and starch) and of lipid contents (overall fatty acid composition and lipid amount) was undertaken during seed development in wild type plant. A first biochemical characterization of the 80 mutant lines has been achieved and a few interesting mutants have been selected. Detailed molecular characterization and cytological analyses are under progress on these mutants.

This work is part of a collaboration with several teams involved in other aspects of the mutants characterization (J. Guéguen, INRA Nantes, proteins; M. Delseny, CNRS Perpignan, storage and LEA proteins; V. Gomord, CNRS Rouen, proteoglycans; J. Giraudat, ISV Gif, transcriptome; T. Barsby, Biogemma UK, lipid enzyme activities).

82 Sannotation: Providing a Unified Whole Genome Annotation of Arabidopsis

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The Institute for Genomic Research

The Arabidopsis sequencing project employed a hierarchical sequencing strategy in which shotgun sequencing was applied to bacterial artificial chromosomes (BACs). Immediately subsequent to the sequencing of each BAC, annotation methodologies were utilized to discover genes and other biological features within the sequence. The annotation methodologies included the use of automated splice site, exon, and gene predictions, in addition to sensitive alignments generated by sequence database searching programs. More accurate gene and splice site prediction programs have become available. Additional sequence information such as genome annotation, EST sequences, and full-length cDNAs, has been deposited in the public archives. As a result of the sequencing and annotation strategy employed, in addition to the evolution of annotation methodologies during the course of the sequencing project, the current state of the Arabidopsis genome annotation is best described as heterogeneous.

The Institute for Genomic Research (TIGR) has been funded to reannotate the Arabidopsis genome, providing the community with an accurate, up to date and unified view of the Arabidopsis genome annotation. The reannotation process involves the refinement of existing gene models, the identification of undiscovered genes, the examination of gene names and the assignment of genes to Gene Ontology role categories. This project will exploit the knowledge gained from the construction and analysis of Arabidopsis gene families and from regions of segmental duplication within the genome. We have also begun the incorporation of greater than 5000 full-length cDNAs into the reannotation pipeline, and strategies using comparative genomics are currently being evaluated for their use in identifying novel genes. Examples demonstrating the utility of TIGRs latest annotation methodologies are provided, and the impact of our latest strategies and collaborations are discussed. This work is supported by the National Science Foundation.

83 The Complete Arabidopsis Transcriptome MicroArray (CATMA) Project

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The CATMA Consortium (http://jic-bioinfo.bbsrc.ac.uk/CATMA/)

The aim of the CATMA project is the design and production of high quality Gene Specific Tags (GSTs) covering most Arabidopsis genes, for use in transcription profiling experiments as well as other functional genomic approaches.

The identification of each gene in the five Arabidopsis chromosomes is at the root of a genome-wide effort to study their expression. Since still only a minority of Arabidopsis genes have been determined experimentally, genome annotation relies on gene prediction to identify the boundaries of each transcription unit and of the exon(s) within it. We have based the selection of GSTs on a novel annotation of the Arabidopsis genome completed with a homogeneous set of parameters.

The Specific Primer and Amplicon Design Software (SPADS) has been developed for the selection of specific regions within genes and the design of primer pairs picked to amplify such regions. Because of the inherent duplicated nature of the Arabidopsis genome, not all genes will be represented by perfect GSTs. But we estimate that 150 to 500 bp genomic fragments will yield gene-specific hybridisation results for over 70% of the SPADS generated GSTs.

The CATMA GSTs are flanked by a limited set of sequences allowing for PCR reamplification and preventing well-to-well cross-contaminations which often plague the storage and dissemination of large-scale clone collections. An exhaustive set of Arabidopsis GSTs, validated in transcription profiling experiments, should be available in early 2002.

84 Glycosyltransferase expression analysis in *Arabidopsis thaliana* using reporter genes

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In plants glycosyltransferases catalyse the addition of a glycosyl group from a Uridine 5-diphospho (UDP)-sugar to a hydrophobic molecule. They are involved in a wide range of biochemical processes such as, anthocyanin biosynthesis, hormone conjugation and detoxification of xenobiotics.

A family of 107 putative UDP-glycosyltransferase (UGT) sequences has been identified from the *Arabidopsis* genome database [1]. In order to further characterise this family, 36 recombinant UGTs have been screened for activity against 11 related phenylpropanoid compounds [2]. The activity of two of these recombinant proteins, UGT72E2 and UGT72E3, towards ferulic acid, sinapic acid, coniferyl alcohol and sinapyl alcohol suggests they may play a role in glucosylating monolignols during lignin biosynthesis. A third recombinant protein UGT84A2 catalysed the production of sinapoyl glucoside from sinapic acid. This UGT may be involved in sinapoylglucose formation *in planta*, both during synthesis of the compound in developing seeds and also in leaves. In leaves sinapoylglucose is converted to sinapoylmalate which acts as a UVB protectant. A second product formed from the glucose ester is sinapoylcholine which is thought to serve as a choline reserve in seeds.

The expression patterns of these three UGT genes are being characterised using promoter-GUS fusions transformed into *Arabidopsis*. Both UGT72E2 and UGT72E3 have closely related sequences and their expressed proteins glucosylate similar phenylpropanoids. However, in the promoter-GUS fusion transgenic plants, differences in gene expression profiles have been observed. In UGT84A2 promoter-GUS fusion transgenic plants, gene expression profiles appear to have broadly similar patterns to UGT72E2 and UGT72E3. Using these transgenic plants and various mutants such as the ferulate 5 hydroxylase mutant (*fah*1), we aim to investigate any possible link between UGT gene expression and lignin deposition.

[1] Li et al. 2001. J. Biol. Chem 276: 4338-4343

[2] Lim et al. 2001. J. Biol. Chem 276: 4344-4349

85 Use of Arabidopsis microarrays for study of gene expression in other plant systems

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Use of *Arabidopsis* microarrays holds great promise for gaining insight and understanding of signal transduction processes in this plant. However, numerous interesting questions such as control of dormancy in vegetative propagules of perennial plants can not yet be addressed in *Arabidopsis*. Sufficient genomic information and resources are not always available for other plant systems to allow microarray analysis. Yet, there is significant conservation in plant genes and regulatory pathways. To determine if there is sufficient conservation between *Arabidopsis* and other plant systems to allow use of *Arabidopsis* microarrays directly, we have screened *Arabidopsis* arrays with probes developed from growing apices and mature leaves from leafy spurge, poplar, and wild oat. Two biological replications of the hybridization were performed on each species. We observed an average of 48%, 55%, and 68% of the genes present on the *Arabidopsis* array hybridized to probes derived from wild oat, poplar and leafy spurge respectively. It is recognized that these values are subject to various factors that may increase or decrease hybridization efficiency. Also, the expression pattern for many of the genes are consistent with conservation of signaling pathways between these organisms. Expression of several conserved differentially expressed genes has been further characterized to confirm microarray expression patterns and to provided insights into the signaling processes controlling bud dormancy in a perennial weed (leafy spurge). Consequently, it appears that *Arabidopsis* microarrays can be useful for identifying differentially expressed genes and signal transduction pathways from diverse plant species.

86 Gene tagging system using *Ds* element: Mapping of *Ds*-insertion sites transposed from start loci on chromosome 5

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Transposons are widely used for gene tagging approach in various organisms not only because they have advantage for gene isolation, but also because they can be remobilized. In order to construct a transposon insertion library, we have used the maize *Ds* element. We have produced *Ds* insertion lines (1) from 3 parental donors *Ds*-T-DNA lines whose loci had been mapped on chromosome 5 (2). Genomic DNA flanking inserts have been amplified by using TAIL-PCR, and the *Ds* insertion sites were identified for about 1,000 lines. About 60% of *Ds* were inserted in the open reading frame (ORF) or in the 5' or 3' 500-bp region of the ORF.

About half of the inserts were mapped on chromosome 5, and insertion hot spots were confirmed at the 3 start loci. Other hot spots were found on north ends of chromosome 2 and 4. *Ds* tends to transpose to chromosome ends and to avoid centromere regions. More than half of the transposed *Ds* did not possess perfect inverted repeat element necessary for transposition, indicating that it is difficult for these lines to use launching pad for transposition. Albino phenotypes of 15 mutants out of 19 mutants were cosegregated with *Ds* insertion and were probably tagged, indicating that this system is good for gene isolation responsible for the mutant phenotype. The insertion site database will be used for *in silico* screening of knockout lines.

(1) Ito *et al., Plant J*. 17, 433. (2) Smith *et al*. *Plant J*. 10, 721.

87 Generation and Characterization of Gene Trap Lines of *Arabidopsis thaliana*

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In order to establish a system for characterizing gene function by utilizing the genome sequence information, we have been generating a large number of T-DNA insertion lines using a newly constructed binary vector according to the *in planta* 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 lines in combination with the *Ac* element, and the *cis* sequences required for *Agrobacterium*-mediated transformation. To date, we have generated a total of 36,000 transgenic lines. Approximately 8% of the 17,000 plants tested for GUS activity exhibited positive staining. The insertion points which provide the donor loci for transposition of the *Ds* elements have been determined by sequencing the flanking regions.

88 GAL4-GFP enhancer trap lines — resources for visualizing and manipulating gene expression in Arabidopsis

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Transgenic lines that express the heterologous transcriptional activator GAL4-VP16 in tissue- or cell-specific patterns have been widely used for regulating gene expression in Drosophila and, more recently, in Arabidopsis. This approach involves fusing the coding region of a gene-of-interest (GOI) to a GAL4 upstream-activating-sequence (UAS), introducing this gene into plants by transformation, and then crossing this UAS::GOI line to a line that expresses GAL4-VP16 in a desired pattern. 5,000 transgenic Columbia lines were generated using an enhancer trap vector containing both a promoterless GAL4-VP16 and the reporter gene UAS::GFP. 3,500 of these lines were screened for GFP expression in the root, shoot, and inflorescence and approximately 250 lines with stable patterns of GFP expression in the F4 generation are currently being characterized. Several interesting expression patterns will be illustrated. DNA sequences flanking the T-DNA insertions have been determined for about 100 of these lines. The ability of these lines to transactivate gene expression has been demonstrated by crossing a subset to a transgenic line carrying UAS::GUS. In addition, we have produced a set of vectors that will facilitate the use of this system for manipulating gene expression, mosaic analysis, genetic ablation studies, and activation tagging mutagenesis.

89 Genomic DNA- vs. RNA-based references in gene expression profiling for the chromosome 2 of Arabidopsis in response to oxidative stress.

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In current protocols, labeled DNA molecules derived from two RNA samples are often co-hybridized to microarrays to measure gene expression specific to the tested conditions, as compared to that in the reference conditions. The use of RNA-based references is generally highly effective in providing reproducible high quality results. However, there are fundamental disadvantages in using such references: they generally fail to hybridize to all the legitimate spot elements on the arrays, and the gene expression patterns generated with different references can not be directly compared to each other. As an alternative to RNA-based references, we tested genomic DNA (gDNA) for its ability to be a reliable universal reference for gene expression profiling, using Arabidopsis as a model system. Co-hybridizations with Arabidopsis gDNA labeled with Cy3 and Cy5 dyes showed a good correlation between the signals, indicating that labeling and hybridization reactions with gDNA are consistent. Moreover, independent hybridizations with a duplicate set of gDNA and cDNA samples produced by oxidative stress responses showed high reproducibility of the experiments that involve the gDNA references. We also compared the gene expression profiles generated from these references to those from the RNA-based reference set. For the most part, both results showed the same response genes, although both allowed identification of additional genes whose expression must be verified. Based on this study, we conclude that gDNA can serve as a reference for reliable gene expression profiling, and thereby it can provide a simple means based on which cross-experiment comparisons of gene expression data become now possible.

90 Structure and Expression of Phosphate Translocator Genes in *Arabidopsis thaliana*

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Plastids contain various proteins that mediate the exchange of metabolites between the stroma and the cytosol. The triose phosphate/phosphate translocator (TPT) represents one of the well characterized transport systems of the inner envelope membrane. In the last years, three other proteins have been isolated from different plants, which are similar to the TPT and thus belong to the family of phosphate translocator proteins: the phosphoenolpyruvate/phosphate translocator (PPT), the glucose-6-phosphate/phosphate translocator (GPT), and the ribulose-5-phosphate/phosphate translocator (RPT). To analyze the structure of these genes, genomic clones were isolated and sequenced. Furthermore all homologous genes and pseudogenes in *Arabidopsis* were identified and a phylogenetic tree has been constructed to reveal the evolutionary relationship of these genes. The expression profiles of the phosphate translocator genes were examined by means of Northern blot analysis, RT-PCR and promoter-GUS fusions.

Literature: Fluegge, U.I. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:27-45 (1999)

91 New protein-protein interactions using a high thruput yeast two-hybrid system

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Protein-Protein interaction mapping using a large-scale two-hybrid screen has been proposed as a way to functionally annotate large numbers of characterized and uncharacterized proteins predicted by complete genome sequences. We have chosen to focus on Arabidopsis in view of the imminent availability of the complete nuclear genome sequence in the public domain. Together with the organelle genomes, this represents the complete blueprint for the entire organism throughout its lifecycle. At the Max-Planck-Institute for Molecular Genetics we have developed an automated yeast two-hybrid system for the identification of protein-protein interactions. Within this method preselection and double counterselection for excluding false positives are combined with robot arraying technology for handling large numbers of yeast clones. At first, the cDNAs of the MSU-library (OHIO stock) were fused to a LexA-DNA-binding domain and preselected by yeast growth and ß-galactosidase activity. These fusion proteins were screened against a Gal4-activation domain Arabidopsis library. The identity of the interacting proteins expressed in individual yeast clones was determined by DNA hybridization, PCR, restriction and sequence analysis of isolated plasmids. We will use our two-hybrid system to investigate the complex network of protein interactions in Arabidopsis thaliana. These investigations are part of a larger plant proteome project involving the characterization of thousands of Arabidopsis proteins on the basis of a broad spectrum of functional parameters.

92 Dissection of chloroplast functions in Arabidopsis by differential expression analysis of 1900 GSTs

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Transcriptomics analyses the expression of genes during different developmental stages or in response to certain stimuli and employs microarrays containing large numbers of DNA fragments as either cDNAs, oligonucleotides or genomic DNA fragments. Higher plant photosynthesis is located in the chloroplast organelle and between 2.500 to 3.100 proteins with chloroplast localisation have been predicted for *Arabidopsis thaliana*. For a functional genomics approach we generated by PCR about 1900 chloroplast-relevant gene fragments for the setup of a “plastid-function” microarray. For this goal a software system was developed to retrieve database information and to design automatically primers suitable to amplify genes encoding chloroplast-targeted proteins. Expression analyses with complex mRNA probes obtained from different conditions were performed and the feasibility of this array for the analysis of chloroplast function will be demonstrated.

93 Tissue Profiling using Arabidopsis Microarrays

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The microarray facility at Michigan State University is part of the Arabidopsis Functional Genomics Consortium (AFGC), a collaboration funded by the National Science Foundation. One goal of this project is to profile gene expression patterns from different tissues to be used as a base set of data for expression analyses. RNA isolated from flower, leaf, root, stem, and silique tissue was compared to a common reference sample on microarrays containing 11,000 ESTs representing approximately 8,000 unique genes. Comparison of these individual tissues to one another showed an extraordinary number of differences compared to other types of microarray experiments with up to 30% of the genes showing differential expression. Approximately 10% of the genes were predominantly expressed in a single tissue with roots showing the largest number of unique genes. Expression data for this subset of genes was analyzed using the GeneSpring program from Silicon Genetics. All data sets are available on the Stanford Microarray Database (Genome-www4.Stanford.edu/MicroArray/SMD).

94 DNA chip analyses of Arabidopsis guard cell gene expression

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Guard cells located within the epidermal cell layer of higher plants form stomatal complexes and are responsible for regulating gas exchange between leaves and atmosphere. Because guard cells integrate hormone and light signals to optimize CO2 influx and water loss affecting whole plant growth and physiology, they have become an attractive plant system to study signal transduction. However, little is known about the regulation of gene expression in guard cells. In order to reveal genes that are expressed in guard cells and their regulation by ABA, we have performed experiments using high-density oligonucleotide gene chips (Affymetrix) representing more than 8000 *Arabidopsis* genes. Using RNA extracted from highly purified preparations of guard cell protoplasts, transcription profiles of *Arabidopsis* guard cells and their responses to ABA were generated. These expression results have been analyzed to identify genes that are both expressed in guard cells and show ABA-regulated expression. The identification and the analyses of these guard cell expressed genes combined with molecular genetic and physiological studies will allow further dissection of molecular events in guard cell signal transduction pathways.

95 New molecular phenotypes in the *dst* mutants of *Arabidopsis* revealed by DNA microarray analysis

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We have used DNA microarrays to study gene expression in the *dst1* mutant of *Arabidopsis*. A novel selection in transgenic plants allowed the isolation of *dst* mutants of *Arabidopsis thaliana* that elevate the abundance of mRNAs that contain the plant mRNA instability sequence called DST. The *dst* mutants offer a unique opportunity to study rapid sequence-specific mRNA decay pathways in eukaryotes. These mutants show a 3- to 4-fold increase in mRNA levels for transgene constructs containing DST elements when analyzed by RNA gel blot; however they show no visible aberrant phenotype. Using microarrays, we were able to identify new genes with altered mRNA abundance in *dst1* in addition to verifying the differential regulation of the transgene constructs used to isolate these mutants. Northern blot analysis was used to confirm the microarray data for some genes and also to study molecular differences in gene expression between *dst1* and *dst2* mutants. These differences revealed previously unknown molecular phenotypes for the *dst* mutants which will be helpful in future analyses. Cluster analysis of genes altered in *dst1* revealed new co-expression patterns that may indicate some clues about the nature of the *dst1* mutation and the role of the DST-mediated mRNA pathway in plants.
Funding provided by: DOE, USDA and NSF

96 FULL LENGTH cDNAs: AN ENTRY POINT TO PLANT FUNCTIONAL GENOMICS

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A full-length (FL) cDNA library construction and sequencing strategy was used to find genes for Arabidopsis, maize and several other plant species and to build an integrated functional genomics platform for Arabidopsis. Tens of thousands of unique full-length cDNAs from Arabidopsis, maize and other species were identified and sequenced. An analysis of the population of FL cDNAs revealed the nature of the UTRs, codon usage and encoded protein products for these species. For example, the second amino acid is Alanine 32 and 40% of the time in Arabidopsis and Zea proteins, respectively. The higher percentage in Zea corresponds to the increased GC content. In addition, maize UTRs are twice as long as Arabidopsis UTRs on average. In Arabidopsis the number of "full-length" cDNAs was increased by using nucleic acid statistics from the population of sequenced FL cDNAs in the design of algorithms and using these algorithms to predict gene structure in genomic DNA, for which FL cDNAs were not sequenced. In addition, FL corn cDNAs were employed to identify the coding regions of Arabidopsis genes. PCR products made from the FL cDNAs and other identified genes were used to build microarrays and to identify genes represented by restriction fragments on comprehensive cDNA-AFLP gels. Gene-specific primers were generated from the cDNAs as well as the predicted genes and used in our highly automated Reverse Genetics program to identify knock-out mutants in a comprehensive collection of T-DNA inserts. The FL cDNAs provided clones for a large fraction of the Arabidopsis ORFs and these are readily added to expression vectors to study the effects of expressing each ORF in specific cells, tissues, organs and environments. Specific examples of discoveries about Arabidopsis genes and transcripts will be given and the efficiencies of the integrated platform based on cDNAs will be described.

97 Construction of an Arabidopsis open reading frame library for functional characterisation of gene families using high-throughput approaches

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The Arabidopsis genome sequencing program has provided the Plant Biology community with the sequence of about 26,000 Arabidopsis genes. For about two-thirds of these genes, some idea of the function of the gene product can be gleaned from sequence similarity to other genes but detailed experimental data are available for only a tiny fraction of these genes. For the other 30% of the genes revealed by genome sequencing, we have no clues to the function of the gene product. Hence there remains a huge amount of work before we understand the function of all of the Arabidopsis genes. To accelerate this analysis, it is crucial to develop high-throughput approaches that allow the functional characterisation of hundreds of genes in parallel.

The purpose of our project is to develop a novel cloning technique to clone thousands of Arabidopsis open reading frames (ORFs) into the expression vectors needed for functional analyses. The Gateway™ cloning system from Invitrogen based on recombinational cloning is used. This system avoids the need for restriction enzymes and ligase and minimises the screening for correctly cloned inserts. In addition, it is ideally suited to automatisation and therefore provides a solution for large scale cloning of ORFs into many different expression vectors. The Gateway™ cloning system has been used recently to characterise hundreds of *Caenorhabditis elegans* protein-protein interactions [1,2]. Furthermore, this system allowed Simpson *et al.* [3] to characterise the intracellular localisation of one hundred human proteins tagged with GFP.

The methods are currently being optimised in the laboratory on the PPR gene family. The PPR family is a large family (about 450 genes in Arabidopsis) of organelle-targeted proteins characterized by the presence of tandem arrays of a 35-amino-acid repeat [4,5]. The function of these proteins is unknown although they are presumed to play various roles in organelle gene expression.

[1] Walhout *et al.* (2000) Science 287: 116-122. [2] Reboul *et al.* (2001) Nature Genetics 27: 332-336. [3] Simpson *et al.* (2000) EMBO reports 1: 287-292. [4] Aubourg *et al.* (2000) Plant Mol Biol 42: 603-13. [5] Small and Peeters (2000) Trends Biochem Sci, 25, 46-7.

98 Identification and Analysis of Arabidopsis ESTs that are characteristic of noncoding RNAs

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Sequencing of the Arabidopsis genome has led to the identification of thousands of new putative genes based on the predicted proteins they encode. Genes encoding tRNAs, ribosomal RNAs and snoRNAs have also been annotated. However, emerging data indicate that one potentially important class of genes has largely escaped detection. These are the genes that correspond to RNAs that lack significant open reading frames and appear to encode RNA as their final product. Accumulating evidence indicates that such "noncoding RNAs" (ncRNAs) can play critical roles in a wide range of cellular processes from protein secretion to gene regulation. Some are naturally occurring antisense RNAs whereas others have more complex structures. Most of the ncRNA genes recognized to date were identified genetically or by accident, although emerging data indicate that systematic searches should reveal many more. As a first step, we collected existing data on Arabidopsis ncRNAs and expanded on this by examining about 20,000 ESTs for characteristics of noncoding RNAs. About 15 putative Arabidopsis ncRNAs have been reported in the literature or have been annotated. Several have homologs in other plants, but all appear to be plant-specific with the exception of SRP RNA. Conversely, none of about 30 ncRNAs reported from yeast or animal systems have homologs in Arabidopsis. To identify additional genes that appear to encode ncRNAs, we used computational tools to filter out the protein coding genes from those corresponding to 20,000 EST clones. What remained were more than 40 clones that either had the characteristics of ncRNAs (19), peptide coding RNAs (pepRNAs)(10) or could not be differentiated between the two categories(10). Again none of these clones had homologs outside the plant kingdom indicating that most ncRNAs of Arabidopsis are likely plant-specific. The ESTs used for this analysis included those on the yr 2000 AFGC DNA microarray (http://afgc.stanford.edu), and many appear highly regulated in specific microarray experiments. These data indicate that ncRNAs represent a significant and underdeveloped aspect of Arabidopsis genomics that deserves further study from multiple perspectives.

[1]This work was supported by NSF and DOE grants to PJG

99 Differential expression of genes caused by the demethylating agent 5-aza-2'-deoxycytidine in Arabidopsis allotetraploids and in the parents A. thaliana and C. arenosa

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The joining of different genomes in allotetraploids played a major role in plant evolution, but the molecular implications of this event are poorly understood. We use synthetic hybrids of *A. thaliana* and *C. arenosa* to investigate the molecular events underlying allopolyploidization. The occurrence of frequent gene silencing, which we previously reported, suggests the involvement of epigenetic phenomena. Changes in DNA methylation were frequent in the recent allopolyploids. Furthermore, treatment of synthetic allotetraploids and parents with 5-aza-2'-deoxycytidine (5-azaC), an inhibitor of DNA methyltransferase, resulted in the development of a homeotic syndrome in the synthetic allotetraploids, but not in the parents. DNA demethylation is known to induce the expression of many suppressed loci, mainly heterochromatic transposons. In certain cases, this response is accompanied by the hypermethylation and silencing of *AGAMOUS* and *SUPERMAN*. We profiled mRNAs in control and 5-azaC treated parents and allotetraploids by AFLP-cDNA and measured the level of specific RNAs by RT-PCR. We found that DNA demethylation induced and repressed two different sets of transcripts. Interestingly, in leaves transcription of *SUPERMAN* was induced by 5-azaC and by antisense RNA suppression of *MET1*, as if DNA methylation played a role in normal regulation. Comparison of the transcriptional response in parents and allotetraploids will be presented and correlated to the phenotypic response induced by 5-azaC. The results are consistent with the hypothesis that these allopolyploids have compromised mechanisms of epigenetic regulation.

100 Analysis of plant responses to fungal pathogenesis using Arabidopsis stress microarrays

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We have constructed an Arabidopsis stress microarray containing approximately 1200 ESTs representing genes that are induced or repressed in response to biotic (virulent and avirulent strains of the bacterium *Pseudomonas syringae* and of the fungus *Peronospora parasitica*) and abiotic (acute ozone, chronic ozone, salicylic acid, jasmonic acid) stresses using PCR-based suppression subtractive hybridization. We are conducting time-course expression profiling experiments using the stress array to study plant-fungus interactions. Arabidopsis ecotype Columbia and *pad4* mutant plants infected with *P. parasitica* strain Ahco2, Ws ecotype infected with *P. parasitica* strain Emwa2 were used for analyzing the compatible interactions. For microarray hybridizations, total RNA was isolated from plants collected at 24-hour intervals for 6 days starting one day after fungal inoculation. Water-treated control plants were harvested for each time-point. The experiments were repeated twice and genes exhibiting greater than two-fold induction or repression and displaying a consistent expression pattern in replicate experiments were subjected to further analysis. Identifying plant genes during the complex interplay between host and biotrophic pathogen will provide vital information about plant components during disease progression. Composite information from expression profiling, sequence analysis and the available biochemical data were used to identify key early stress response signaling and regulatory genes. These predictions are being tested by creating mutants using dsRNA or antisense constructs to identify subnetworks of regulatory genes and the cognate effector genes.

101 Validation of Hypothetical Gene Predictions in Arabidopsis thaliana by Full-length cDNA Cloning

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Analysis and annotation of Arabidopsis thaliana genome has revealed that about 25% of the genes are supported only by computational predictions with no similarity to known proteins or EST sequences. Many of these "hypothetical proteins" are closely related to sequences found elsewhere in the genome. We have begun to construct and analyze protein families containing "hypothetical proteins" with a view to experimental verification of the genes and their structures. A partial proteome consisting of 14,767 proteins was analyzed and clustered into families using the domain-based GEANFAMMER package. This analysis focuses on "hypothetical proteins" from chromosome 2 which was completely sequenced and annotated at TIGR. The goals of this research are to demonstrate that the predictions of hypothetical genes are valid and that the genes are actually expressed, and to elucidate the correct gene structure. The total number of predicted genes on chromosome 2 was 4037, out of which 1094 were hypothetical (Lin et al, Nature 402:761-768, 1999). Data released since that time has reduced the number of hypothetical proteins (genes with no GenBank support) to 847. Out of 847 hypothetical genes, 206 are members of gene families and 641 are singletons. We are using 5' and 3' RACE to isolate full-length cDNAs for these hypothetical genes. As template, we employ mRNA isolated from a variety of tissues/treatments (e.g. callus culture cells, cold, heat, pathogen treated plants), to increase the probability of amplifying the transcript. The 5' and 3'-RACE products are sequenced and aligned with genomic sequence to generate the correct gene structure. So far we have examined 24 genes representing 13 gene families. Fifteen of these 24 have been successfully cloned; the other 9 genes are not expressed in any of the six RNA populations tested. Of the six genes sequenced to date, five confirm the predicted gene structure while sequence for one gene indicates that the gene model should be modified. The results from these analyses will be presented. This research was supported by NSF.

102 New Methods for SNP Determination and DNA Isolation

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Promega Corporation

Rapid improvement in plant species often involves the transfer of a specific allele from one plant strain into another strain. Identifying offspring from such a cross with the desired genotype requires that allele determinations be performed on a large number of plants. However, isolating DNA from plants can be a cumbersome task often involving organic chemicals. In addition, allele determination is often limited by the use of either gel based systems or the high cost of equipment or modified probes required by high throughput methods. This presentation describes the combination of two new methods that simplify genotype determinations.

The first method allows high quality DNA isolation from plants using MagneSil™ paramagnetic particles. The use of such particles allows the purification of the DNA to be performed in a manual method or with the assistance of automated equipment. The purification methodology has been used with a wide range of plant tissue including leaf, stem, root and seed. The second system, the READIT™ SNP Genotyping System, is used to perform genotype determinations on the purified DNA following amplification of the desired target sequence. The genotyping system utilizes a series of coupled enzymatic reactions to generate a light signal dependent upon the sequence of the samples interrogated. This system also allows the user to match throughput needs to equipment available. Together these systems provide users with the ability to analyze a very wide number of samples for all types of genetic alterations.

103 T-DNA Insertional Mutagenesis of Genes Required for Seed Development

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The purpose of this project was to identify large numbers of *Arabidopsis* genes with essential functions during seed development. More than 120,000 T-DNA insertion lines were generated following *Agrobacterium*-mediated transformation. Transgenic plants were screened for defective seeds and putative mutants subjected to detailed analysis in subsequent generations. Plasmid rescue and TAIL-PCR were used to recover plant sequences flanking insertion sites in tagged mutants. More than 4,200 mutants with a wide range of seed phenotypes were identified. Over 1,700 of these mutants were analyzed in detail. The frequency of multiple insertion sites and percentage of mutants tagged with T-DNA are consistent with results from other populations. The 350 tagged embryo-defective (*emb*) mutants identified to date represent a significant advance towards saturation mutagenesis of *EMB* genes in *Arabidopsis*. Plant sequences adjacent to T-DNA borders in mutants with confirmed insertion sites were used to map genome locations and establish tentative identities for 167 *EMB* genes with diverse biological functions. The frequency of duplicate mutant alleles recovered is consistent with a relatively small number of essential (*EMB*) genes with non-redundant functions during seed development. Other functions critical to seed development in *Arabidopsis* may be protected from deleterious mutations by extensive genome duplications.

104 The Arabidopsis Knockout Facility

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The availability of a mutant line in which the action of a known, specific gene has been disrupted gives the plant biologist a powerful tool in understanding the action of that gene. Insertional mutagenesis, using t-DNA from *Agrobacterium,* can be used to create a large population of plants containing randomly inserted pieces of foreign DNA. If the sequence of a gene is known, it is possible to devise a PCR-based strategy to identify a plant where that specific gene has been disrupted by the insertion of foreign DNA. To fully utilize this technology, it is necessary to saturate the genome with insertion mutations, and to develop efficient PCR-based screening methods to comb through knockout plant populations and identify specific mutant plants. Using an initial population of 60,480 lines, PCR methodology was developed to efficiently screen pooled DNA samples for specific mutant lines. As part of the AFGC, an Arabidopsis Knockout Facility was established at UW-Madison in October 1999 to give researchers worldwide access to this technology and to the initial population of mutant lines. Basically users design primers to their specific gene and the Facility performs two rounds of PCR reactions using these primers and pooled DNA. The first round screens the entire population and the second narrows down the hit to a particular subset of the population. Another population of 72,960 lines was produced and made available in November 2000. To date over 500 PI's have used the Facility and over 2500 first round and 1800 second round screens have been performed. Approximately 55-60% of screens identify a knockout plant. Full details of the populations and how to access the Facility are given at http://www.biotech.wisc.edu/Arabidopsis

105 A Comparison of SAGE and DNA Microarrays - Large Scale Analysis of Gene Expression in Arabidopsis Overexpressing the Tomato Pti4 Gene

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Serial analysis of gene expression (SAGE) and DNA microarrays are two powerful genome-wide methods for quantification of transcript levels. SAGE has been widely used for absolute and comparative analyses of gene expression. One challenge in applying the SAGE method to a new organism is to assign SAGE tags to specific genes. We will present the development of a SAGE map for *Arabidopsis*. DNA microarrays provide a measure of the relative abundance of transcripts in the two samples hybridized to the same slide. However, only genes represented by target DNAs on the microarray slides can be monitored. In contrast, the SAGE method provides a measure of the frequency for all individual transcripts, a measure reflecting the transcript abundance in the RNA sample. Our goal was to compare results obtained from these two methods so as to better understand their strengths and weaknesses. The source material for this experiment was RNA from *Arabidopsis* plants overexpressing the putative transcription factor *Pti4* from tomato and wild-type Columbia plants. The PTI4 protein was identified by its interaction with the bacterial speck disease resistance protein, PTO. PTI4 possesses a highly conserved DNA-binding domain that recognizes a core sequence found in the promoters of several genes that encode ethylene-induced defense-related proteins (e.g. pathogenesis-related proteins). This comparative experiment is one component of a larger project to determine down-stream targets for PTI4. From a total of 27,000 SAGE tags (13,500 per genotype), 400 genes were identified as differentially expressed in the *Pti4* transgenics relative to the wild-type. Plant material collected under the same conditions will be used to probe the AFGC DNA microarrays (http://afgc.stanford.edu), which contain ~11,500 cDNA targets. A comparison of the differentially expressed genes identified with DNA microarrays and SAGE will be presented.

106 A High Throughput Targeted System for Altering Gene Expression in Arabidopsis

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The successful characterization of gene function often requires identification and characterization of a mutant plant with an altered gene expression. As a key step in our industrialized gene function discovery platform, we have developed a high throughput system for up- or down-regulating specific genes in *Arabidopsis*. The system consists of a transgene containing a DNA binding domain and a transcriptional activation domain (**Driver**), and a transgene containing the gene of interest (**Target**) cloned in sense or antisense orientation behind a minimal promoter and upstream activating sequences (UAS). The **Driver** transgene is required to activate expression of the **Target**. Results will be presented to demonstrate the spatial and temporal expression patterns of our transactivator and the utility of our system in deciphering the role several unknown genes. Further, the versatility of our system to include tissue specific promoters and target transgene expression to specific plant organs, will also be discussed.

107 The extent of linkage disequilibrium in the highly selfing species *Arabidopsis thaliana*

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Linkage disequilibrium (or LD) – the non-random occurrence of alleles in haplotypes – has long been of interest to population geneticists. More recently, the rapidly increasing availability of genomic polymorphism data has led to intense interest in LD as a tool for fine-scale mapping, in particular of human disease loci. The chromosomal extent of LD is of crucial importance in this context, because it determines how dense a map must be for associations to be detected, and, conversely, limits how finely loci may be mapped. *Arabidopsis thaliana* is expected to harbor unusually extensive LD because of its high degree of selfing. Several polymorphism studies have found extremely strong LD within individual loci, but also evidence of some recombination. Here we investigate the pattern of LD on a genomic scale, and demonstrate that in global samples, LD decays over approximately 1–2 cM, or 250–500 kb. We also show that LD in local populations may be much stronger, presumably as a result of founder events. The combination of a relatively high level of polymorphism and extensive haplotype structure bodes well for the prospects of developing a genome-wide LD map in *A. thaliana*.

108 Proteomics of Arabidopsis thaliana chloroplasts; prediction, experimentation and protein-protein interactions

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A combined experimental analysis and genome-wide prediction of the proteome of the thylakoid lumen of chloroplasts in A. thaliana will be presented. Thylakoid lumen proteins were purified, separated by denaturing 2-DE gels and identified by matrix assisted laser desorption mass spectrometry and nanospray tandem mass spectrometry. Gene annotation of numerous lumenal proteins in the A. thaliana database was corrected by sequence tags obtained by mass spectrometry and by comparison with overlapping EST's. A number of small protein families, as well as a surprising number of isoforms were detected and are discussed. Information from the experimentally determined lumenal proteome was used to provide parameters and cut-off values for a genome-wide theoretical prediction of the thylakoid lumenal proteome, using modified versions of the cellular localization programs TargetP and SignalP, combined with threshold and patterns obtained from the experimental set of lumenal proteins. Ca 100 proteins translocated through the TAT pathway were identified and it is estimated that the lumen contains maximally ca 250 proteins. In addition, we report the identification of a 350 kDa ClpP protease complex with 10 different subunits in chloroplast of A. thaliana using BN-PAGE, followed by mass spectrometry. The complex contains chloroplast-encoded pClpP and six nuclear-encoded proteins nCpP1-6, as well as two unassigned Clp homologues (nClpP7, nClpP8). An additional Clp protein was identified which does not belong to any of the known Clp genes families and is here assigned ClpS1. Expression and accumulation of several of these Clp proteins have never been shown earlier. The putative funcationality and location of the different isoforms is discussed

109 A transposon-based method for creating deletions using Cre-*lox* site specific recombination.

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We have constructed a transposon system based on the maize *Activator-Dissociation* (*Ac-Ds*) transposon to create small deletions and inversions in the *Arabidopsis* genome. For this purpose, we have incorporated *loxP* recognition sites for the Cre-recombinase into the donor T-DNA and into the transposon. The donor site T-DNA contains three selectable markers, a chlorosulfuron-resistant *ALS* gene, an *nptII* gene conferring kanamycin resistance and the bacterial cytosine deaminase (*codA*) gene as a negative selectable marker. Plants expressing this gene can be killed by 5-fluorocytosine (5FC). The *codA* gene allows identification of linked transposition events, as well as inversions and deletions on either side of the donor T-DNA. The *loxP* sites have been inserted in both orientations between the *codA* gene and its promoter in the T-DNA and in the transposon flanking the hygromycin gene. Inversions and deletions extending in either direction from the donor T-DNA will separate the *codA* gene from its promoter, resulting in 5FC resistance. The T-DNA contains the *ALS* gene at the right border and the *nptII* gene at the left border. Both the *ALS* and *nptII* genes persist following an inversion, but one of the two will be eliminated by a deletion. We have identified several lines with transposed elements at distances of between 6 and 500 kb from the donor T-DNA and will report the results of analyses carried out on progeny resulting from crosses to plants carrying the Cre-recombinase gene.

110 MAtDB - connecting sequences to plant lifestyles

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MIPS/IBI at the national research center for environment and health (GSF)

The challenge of the post-genomics era is the integration of biological knowledge and genomic data to build connections between plant lifestyles and genome sequences. The MIPS goal is to model all interactions and pathways in a computable form that will allow classification and the development of prediction methods.

MAtDB (http://mips.gsf.de) goes beyond being a genome sequence database by including functional, expression and phenotypic data. MIPS connects both intrinsic and extrinsic data with every gene sequence. Intrinsic data is data extracted directly from the primary sequence and may be manifested as known gene regulatory elements or protein domains. Extrinsic data is data derived by association or from experimentation. PEDANT (http://pedant.gsf.de) automates a suite of bioinformatic analysis tools and provides state-of-the-art analysis of protein sequences. Additional information is manually annotated.

The MIPS functional catalogue is an ontology that allows hierarchical classification of protein function. The expression data is currently restricted to cognate ESTs, but tools to integrate expression profile data are implemented. The phenotype data is classified e.g. according to developmental stages, cell-types or by physiological process affected.

The underlying database uses GAMS (Generic Annotation Management System) to model the genetic elements and their biological significance and interactions. GAMS is an object-oriented approach to describing genetic data and allows mapping of interactions and parent-child relationships such as gene-mRNA-protein-complex.

The resulting MAtDB data resource is used as a backbone for the representation and comparison of data from other plants especially crops. In the GABI project (www.gabi.de) MIPS functions as a bioinformatics centre and correlates sequence data from plant species as diverse as sugarbeet and barley to Arabidopsis, in this way transferring knowledge across species.

Presently a major focus at MIPS is to implement data interfaces that facilitate integration of knowledge into MAtDB from specialists e.g. on receptor-like kinases, transmembrane proteins and resistance genes.

111 Microarray analysis using genomic DNA as a common reference

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Global changes in gene expression between two samples using cDNA microarrays can be measured directly on one array, using a different fluorescent dye for each sample. When more than two samples need to be directly compared then performing all the pair wise experiments demands an increasing number of arrays. For example, 4 samples would require 6 arrays and 5 samples would require 10 arrays. To reduce the number of arrays needed, each sample can be compared to a common reference, making them directly comparable to each other. An ideal common reference for multiple experiments would be identical in each preparation and would hybridise to every spot on the array. Genomic DNA would meet both these criteria. We used Accession Columbia 0 (Col-0) as a reference tissue to measure changes in gene expression between two different time points during the day (at dawn and 12 hours later at dusk). Six Arabidopsis Functional Genomic consortium (AFGC) 11,000 spot arrays were used for each time point and these were compared to results using a direct comparison. In addition statistical analysis of gene expression values was conducted. Using a students t-test, 692 ESTs showed 99% probability of differential expression. This compares with taking an arbitrary cutoff value of 2 fold or 3 fold which identifies 368 clones or 123 clones respectively. Experiments are currently underway to validate these results.

112 High throughput identification of T-DNA insertion site sequences in Arabidopsis.

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Ceres, Inc.

The availability of the complete *Arabidopsis thaliana* genomic sequence enables new approaches to develop tools for Arabidopsis functional genomics. Loss-of-function mutations are a key resource for understanding the role of a gene in a biological process. Reverse genetics strategies for identifying T-DNA and transposon insertions are well developed. However traditional PCR screening methods using pooled plant/DNA samples, while effective, are limited by throughput. DNA sequence based approaches enable direct localization of an insertion sequence (IS) to a particular genomic location and prediction of possible effect on a target gene's function. Several methods have been used to isolate DNA sequences at the site of IS insertion in a chromosome including IPCR, adaptor/vectorette PCR and TAIL-PCR. Ceres has developed a high throughput platform for the rapid isolation of T-DNA insertion sites in transformed Arabidopsis. The program uses an integrated sequence determination and analysis pipeline in combination with a modified TAIL-PCR procedure to allow researchers to begin their reverse genetics screen *in silico*, using BLAST or a genome annotation viewer. We have used the TAIL platform to analyze a pilot population of 30,000 independent transformants for left border insertion sites. A more limited analysis of right border sequences has also been performed. We will present methods and analysis of the insertion site frequency, genomic distribution, rearrangements and chromosomal location with respect to Ceres full-length cDNAs and annotated coding sequences.

113 Integration of ABRC Information and Order Processing into the TAIR Database

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The Arabidopsis Biological Resource Center (ABRC) and The Arabidopsis Information Resource (TAIR) have established a collaborative effort which will enable ABRC's stocks to be searched and ordered using TAIR. ABRC's data activities have been coordinated through the AIMS database at Michigan State University managed by Dr. Sakti Pramanik. Now all of ABRC's informatics functions will be assumed by TAIR. The development of the informatics necessary to achieve this aim is being assumed by the National Center for Genome Resources (NCGR). This transition is being undertaken so that the sequence, mapping and overall genomics data and capabilities of TAIR can be utilized by the community to efficiently locate clones and strains of interest for ordering in a straightforward, streamlined fashion. Ordering, stock information and automated community addition/updating functions are currently being developed in TAIR, including improvements in the ordering process. The access of ABRC-related functions for users will be implemented in two phases. First, a basic ordering system for all stocks, DNA stock data integration to genomic information in TAIR's database, and a sophisticated interactive community data input system will be installed in summer, 2001 at which time the AIMS site will no longer be used. Ordering through TAIR will involve user login, searching for stocks and placement of orders through the TAIR Web site. The second, complete, phase will be installed in late 2001 and will involve full integration of all ABRC information into TAIR'S database and streamlining the ordering and invoicing processes. In this full version, all functions will be Web based, sophisticated searches for stocks will be supported and all existing ABRC data including stock order histories will be integrated into the system. ABRC and TAIR are supported by grants from the National Science Foundation.

114 Development and Distribution of Stocks at ABRC Relevant to Genomic Studies

Randy Scholl, Doreen Ware, Emma Knee, Deborah Crist, Luz Rivero, Jeff Cotrill and Staci Putney

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The Arabidopsis Biological Resource Center (ABRC) maintains many stocks relevant to genome exploration. Among these, T-DNA lines, associated DNA pools, the BAC genomic clones utilized for the sequencing projects and the EST collection are critical in terms of the expanded utilization by researchers, the completion of genome sequence and the current emphasis on functional genomics. Populations of T-DNA transformants, representing 230,000 total lines, have been received by ABRC from many sources. Some T-DNA lines have been donated in quantities which can be distributed immediately. Currently, pools representing 130,000 of the above lines exist in quantities large enough for community-wide distribution as seeds for forward genetic screening, and 100,000 additional have been or are being grown and/or prepared at ABRC for distribution. The T-DNAs employed to generate these lines include enhancer trap, activation tagging and over-expression constructions, as well as simple insertions. DNA of 12,000 T-DNA lines have been available for some time. Laboratories utilizing the present T-DNA pools were surveyed to ascertain their degree of success with these populations, and the results will be presented. The ABRC is presently expanding its holdings of these isolated DNAs, so that DNA from populations totaling at least 50,000 will be available soon. Cooperation with the Arabidopsis Knockout Facility (AKF) at the University of Wisconsin has also been in progress for some time. ABRC distributes the followup seeds associated with their PCR service, and additional collaborations are planned to expand the public reverse genetic services for the Arabidopsis community. Additional ESTs have been received from C. Benning, which will significantly enhance the representation of the collections. Additional full-length cDNAs are being received, including those from J. Ecker/A. Theologis/R. Davis project. The BAC collection at ABRC is being updated. The ABRC is supported by a grant from the National Science Foundation

115 New Stocks Being Added to the Collection at ABRC

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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 has previously published stock information in the AIMS database. Beginning in summer, 2001 informatics activities of the Resource Center will be assumed by The Arabidopsis Information Resource (TAIR, http://www.arabidopsis.org) with informatics support from the National Center for Genome Resources (NCGR).

Diverse, new stocks have been added to our collections in the past year, including: A) many new mutant lines, B) Pools of T-DNA lines, so that the total lines available is near 230,000, C) characterized insertion lines including GFP and sequence-tagged lines, C) a recombinant inbred population, D) ecotypes from new locations, E) clone accessions, D) libraries, E) full length cDNA clones, and F) isolated DNA from T-DNA populations.

ABRC is striving to fulfill as completely as possible a number of objectives: a) Enhance the collection of characterized mutants and clones to reflect to the maximum extent the published mutants and clones; b) Offer insertion populations that represent as nearly as possible a genome-saturating set of insertions and comprise diverse types of functional insertions; c) Make available various advanced seed resources arising from genome projects, including sequence-tagged insertion mutants; d) Incorporate a complete set of full-length cDNA clones; e) Organize and confirm the identity of the genomic clones related to the published genome sequence data; f) Incorporate enhanced-function clone collections from genome and other projects such as transformable BACs, and incorporate additional genome-related seed and DNA resources as they are developed.

During the past year, ABRC distributed approximately 51,000 seed and 25,000 DNA stocks to researchers. ABRC is supported by the National Science Foundation.

116 Expression Analysis of a UDP-glycosyltransferase Gene Family in *Arabidopsis thaliana* Utilising Microarray Technology

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Glycosyltransferase enzymes are responsible for transferring a sugar molecule from an activated donor to a receptor substrate. Research indicates that one family of these enzymes may be involved in multiple biochemical processes within the plant, including detoxification, transportation, storage and biosynthesis. By screening the *Arabidopsis thaliana* genome database with a peptide motif representing a Uridine Diphospho (UDP)-glucose binding domain, our studies have identified a putative UDP-glycosyltransferase (UGT) gene family containing 107 members (Li et al. 2001).

A multi-disciplinary approach is being adopted for characterising this *Arabidopsis* gene family. As part of this investigation, microarray analysis has been chosen in order to gain an expression profile of all the UGT genes simultaneously. The UGT family members are closely related with DNA sequence identities up to 95%. Various techniques are therefore being investigated to try to distinguish between the UGT genes. These include computer programs designed to identify gene specific regions for PCR fragment arrays, and the use of oligo-arrays. Provisional data is being collected and validated by a Reverse Transcription Polymerase Chain Reaction (RT-PCR) approach, which is utilising an additional computer program to ensure optimum and specific primer design within the gene family.

Using these microarrays, we are currently investigating tissue specificities and the effect of wounding on expression of these UGTs. We also aim to look into other treatments such as pH, light and salinity changes.

Yi Li, Sandie Baldauf, Eng-Kiat Lim, and Dianna J. Bowles; "Phylogenetic Analysis of the UDP-glycosyltransferase Multigene Family of *Arabidopsis thaliana*" J. Biol. Chem. 2001 276: 4338-4343.

117 GARNet, the Genomic Arabidopsis Resource Network.

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GARNet, the Genomic Arabidopsis Resource Network is a UK based functional genomics network created to make full use of the benefits derived from the Arabidopsis Genome Initiative. As part of the BBSRC (the UK Biotechnology and Biological Sciences Research Council) IGF (Investigating Gene Function) initiative, GARNet will be an international facility providing tools and services for functional genomics, which is open for applications from all countries. The produced data will be held in public databases held at NASC and the JIC. GARNet received BBSRC funding for a three years period to establish the services. After this, the services will continue on a cost recovery basis. GARNet is now in its second year.

Within the GARNet project, eight different groups, based at different locations in the UK will provide services or create different resources of functional genomics. The spearpoints of GARNet are metabolite analysis, proteome analysis, micro array technology combined with database mining and bioinformatics and forward and reverse genetics: (large insert) binary clone library screening, high copy transposon insertion mutagenesis, sequencing of transposon insertion sites. Co-ordination of the project is carried out at the University of York.

During the 3 years in which GARNet is funded by the BBSRC, an annual functional genomics meeting will be held at the University of York. The 2001 meeting will take place on September 27 and 28.

Further information on GARNet can be found at the GARNet website at http://www.york.ac.uk/res/garnet/garnet.htm.

118 COMPARISON OF DIFFERENT GENE-FOR-GENE INTERACTIONS IN *ARABIDOPSIS* BY EXPRESSION PROFILING USING OLIGONUCLEOTIDE GENOME ARRAYS

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Plant disease resistance (*R*) gene products specify recognition of avirulence (*avr*) gene products from pathogens and initiate a series of defense responses, including the hypersensitive response (HR), to control pathogen infection. There are multiple gene-for-gene relationships in plants and roughly 0.6% of all the genes in *Arabidopsis* (~168) encode NBS-LRR proteins that resemble the largest class of currently known *R* gene products. In order to identify common and different components among the defense responses elicited by different *avr*/*R* gene interactions, and to relate the structures of different *R* gene products to the downstream signaling pathways that they activate, we are studying the mRNA expression profiles in defense-activated plants. To initiate this work, wild-type *Arabidopsis* plants (Col-0 ecotype) were inoculated with *Pseudomonas syringae pv. tomato* DC3000 expressing *avrRpt2*, *avrRpm1*, *avrPphB* or *avrRps4*, and changes in mRNA expression levels were monitored using Affymetrix GeneChip Arabidopsis Genome Arrays. Reproducibility was examined by doing the above experiments twice. In addition, the same *avr*/*R* pathways were also examined by challenging the plants with non-virulent *P. syringae pv. glycinea* strains expressing the above *avr* genes. Initial analyses of our data sets revealed genes that are regulated only for a particular *avr*/*R* interaction, and genes that are regulated in common for multiple *avr*/*R* interactions. Some of these genes have been previously suggested or shown to be involved in disease resistance, but most of them are either implicated in previously unrelated biochemical processes or are totally unknown genes.

119 Software and Database Design Considerations Utilized In Developing TAIR

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The Arabidopsis Information Resource (TAIR) (http://www.arabidopsis.org) is an information storage and retrieval system designed to provide internet access to all public information pertaining to and derived from the Arabidopsis genome project, in-house analysis and data from the Arabidopsis literature. Central to the development of TAIR is the design of a database system sensitive to considerations for handling biological data. We incorporated an object-oriented design that takes its inspiration from the Object Management Group's (http://www.omg.org/) proposed standards for biological sequences and genomic maps. Browser interfaces into the TAIR database incorporate Java Servlets and Java Server Pages (JSP). The data model was designed with on object-oriented paradigm that efficiently provides a data object pipeline between the Java Servlets/Java Server Pages and the TAIR database. We present our incorporation of a hierarchical database design utilizing subtype relationships that efficiently allow for the compartmentalization of similar biological data types (e.g. genes, markers and clones) while enhancing the association of attribution, keyword, community and reference data with the biological entities.

120 An integrated approach to transcription factor functional genomics

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Transcription factors are central components in the regulation of many processes in plant biology. Transcription factor families are defined by a conserved DNA binding domain that is shared by the members of a given family. Some of these families, such as R2R3-MYB domain proteins, are thought to be specific to plants. The availability of the (nearly) complete *A. thaliana* genome sequence allows assessing the gene inventory of a plant in its entirety. This is especially important for the analysis of gene families because related factors may have overlapping or redundant functions. Despite the long history of studying transcriptional regulation in plants, there is, however, little functional information for the majority of these genes.

Current research in the lab is aiming at the functional dissection of the role of plant transcription factors. Recent studies have shown that plant transcription factor gene families are surprisingly large. For example, the *R2R3-MYB* gene family consists of at least 125 members. A transcription factor cDNA macroarray for parallel gene expression monitoring was generated. This TF array contains about 250 PCR products specific for R2R3- and R1R2R3-MYB, bZIP, and bHLH gene family members. The goal is to determine which TF genes show a change in expression level in response to specific stimuli and/or are differentially expressed in specific plant organs. These data allow to define areas of action of specific TFs and will help to assay for phenotypic changes in the corresponding mutants. To get access to these mutants, FSTs (sequences flanking the insertion sites of T-DNA) are generated from a T-DNA-mutagenised *A. thaliana* population of 70.000 lines.

121 DNA microarray analysis of Arabidopsis flower development

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We are interested in the differences in gene expression between different floral organs and different floral stages as well as in the gene expression networks that control and execute the process of *Arabidopsis* flower development. In order to study flower development on a genomic level we have constructed a cDNA microarray that is composed of flower specific transcripts. DNA microarray technology is a centerpiece of functional genomic studies, which allows the parallel monitoring of the expression of thousands of genes. The *Arabidopsis* cDNA collections that are publicly available have been mainly prepared with mixed RNA populations from the different tissues of the plant. Therefore, these collections are not ideal for constructing a microarray to study flower development, since genes that are specifically expressed in meristems and flowers are under-represented. In order to increase the representation of genes that are involved in the processes we are interested in, we have generated subtracted flower-specific cDNA libraries. We have processed about 7,700 clones from these libraries for printing of the cDNA microarray. In addition, we have specifically cloned or obtained cDNA fragments of approximately 250 genes that are known, or suspected, to play a role in flower development. Furthermore, the microarray also comprises about 100 elements representing constitutive and control genes as well as sequences that can be spiked in the experimental samples to monitor the performance of the technique. The resulting microarray with 8,096 elements was recently completed by the addition of 2,700 flower specific cDNA clones from the non-redundant Kazusa *Arabidopsis*-EST collection. We are currently using the cDNA microarray to analyze the spatial and temporal differences in gene expression during flower development. Furthermore, we are trying to identify the target genes of several of the many transcription factors that have been shown to be involved in the regulation of flower development. Preliminary results of these experiments will be presented.

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122 The AFGC microarray facility at Michigan State University.

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The microarray facility at MSU is part of the Arabidopsis Functional Genomics Consortium (AFGC, http://AFGC.stanford.edu), a collaboration funded by National Science Foundation. Together with the groups of Shauna Somerville at Carnegie Institute and Mike Cherry at Stanford University we have made DNA microarray technology available to the academic community. This has been accomplished through the establishment of a service facility and by providing the data generated to the community through the Stanford Microarray Database (SMD). To date AFGC has received applications for 86 experiments from 79 customers and has performed 61 experiments. Together with the in-house AFGC scientific experiments there are now data in SMD from 183 experimental slides and another 120 slides used for reproducibility tests and the evaluation of labeling technologies. The first AFGC array contained 11,400 DNA fragments, which have been amplified from the collection of MSU EST clones sequenced by Tom Newman (Newman et al. 1994). For the second generation of the AFGC array a collection of ~7,400 unique clones from the original array were supplemented with ~2,000 new seed ESTs (White et al., 2000), ~1,800 other new MSU ESTs and ~3000 GSTs amplified from genomic DNA using custom primers. Thus, ~14,000 unique genes are represented on the second-generation cDNA array which will be used for subsequent AFGC full service experiments.

123 Analysis of Rubisco Small Subunits in Arabidopsis

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Rubisco determines the rate of photosynthesis and thus sets the limit to the size of biosphere. Rubisco is consisting of eight catalytic large subunits and eight small subunits (SSU) of unknown function. The objective of this study is to characterise the rubisco small subunits, which have been difficult to analyze as closely related members of a multigene family. In order to overcome the technical difficulties, a new gene expression assay has been developed. With this assay, we monitored the relative and total levels of SSU gene expression on different environments and correlated them with their relative protein levels. Based on our observations, it seems that a regulatory process has been adopted consisting of closely related SSU genes, which have different sensitivities against different environmental cues. In particular, by changing the SSU gene expression patterns, and thus by changing the relative protein levels, plants might achieve an optimised SSU composition within an rubisco enzyme. In order to verify the hypothesis, protein characterisation is underway using different plants including mutants isolated from reverse screening.

124 A Genome Approach To Mitochondrial-Nuclear Communication In Arabidopsis

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Mitochondria depend on the nuclear genome to encode the vast majority of their proteins, in turn they control the expression of certain nuclear genes to maintain proper functioning. In this work, Arabidopsis leaves were employed as a model to study nuclear gene expression in response to inhibition of the mitochondrial electron transport by antimycin A. Microarrays containing 11,514 Arabidopsis expressed sequence tags supplied through the Arabidopsis Functional Genomics Consortium (AFGC) were used. Transcript levels of 579 nuclear genes were increased at least 2-fold, and the levels of 584 nuclear genes were decreased at least 2-fold after antimycin A treatment. While functions of a large number of the gene products are unknown, others are involved in diverse metabolic activities such as phosphorylation, transcription, and energy metabolism. Data from microarray experiments were repeatable and were confirmed by northern hybridization for specific test genes. It was found through cluster analysis that plant cells show significant common response to chemical inhibition of mitochondrial function, aluminum stress, cadmium stress, hydrogen peroxide, and virus infection. The results imply that these stresses may act on mitochondria and the responses are in part mediated by mitochondrial-nuclear communication. Most nuclear encoded respiratory genes involved in the TCA cycle, electron transport, and ATP synthesis did not respond to signals from the inhibited mitochondria, while genes for cytochrome c and alternative oxidase were induced. The result indicates that these two genes may be targets in the transcriptional regulation of the two respiratory pathways. Custom-made microarrays containing cDNAs that are potentially involved in mitochondrial-nuclear communication are used to further investigate the roles of mitochondria in stress response.

125 High Throughput Functional Genomic Analysis of Arabidopsis Ecotypes

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With the recent completion of the Arabidopsis genome sequencing project the next challenge for plant biologists is the large-scale determination of the function of all the genes required for normal plant growth and development. It is equally challenging and important to study gene function in plants growing under environmental stress conditions so that all genes associated with plant adaptation to environmental stresses can be identified. To meet this challenge, we have developed a high throughput phenotypic analysis platform in which data from over 100 traits representative of all stages of Arabidopsis growth and development under standard and stress environmental conditions are collected using an industrialized process. The high throughput design of the platform allows for the rapid accruement of vast amounts of information in fairly short time. Plants are grown to full maturity in custom walk-in growth rooms or for only few weeks on artificial media in Petri plates positioned vertically in growth chambers. Data are collected at a series of experimental workstations. Each workstation is optimized for a different data collection activity and the plants pass from one station to the next during the data collection process. Plant and sample tracking, as well as the steps in the data collection process, are controlled with a customized laboratory information management system (LIMS). We will present data characterizing the growth and development of a variety of Arabidopsis ecotypes under standard and stress environmental conditions using our high throughput platform. These data establish the capability of our phenotypic analysis platform to reveal the significant differences between genetically distinct lines under both standard and non-standard environmental conditions.

126 The participation of ABA in the glucose-mediated regulation in Arabidopsis

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Sugars act as signalling molecules regulating a variety of metabolic and developmental processes in plants. The evidence so far available points that sugar-mediated regulation in plants, occurs through different signalling pathways. To define the components of the sugar signalling pathways, a number of Arabidopsis glucose insensitive (gin) mutants have been isolated. The characterization of such mutants (mainly *gin5* , *gin6*) has permitted to propose the participation of the ABA hormone as an essential element of the glucose signalling cascade (Arenas et al.,2000). In addition, we have identified a transcriptional factor ABI4, required for proper glucose responses in seedlings. This gene corresponds to an allele of the previously identified *ABI4* gene, involved in ABA response (Finkelstein et al., 1998). To understand the role of ABI4 in the glucose-mediated signalling process, we have followed its expression and regulation under different glucose conditions and in different gin mutants. We have found that the expression of the ABI4 gene is regulated directly by glucose but not by ABA exogenous application in seedlings. We also showed that transcript level of *ABI4* is affected in different of our gin mutants but not in mutants that affect exclusively ABA responses such as *abi1* , *abi2* and *abi3* . Exogenous ABA application restores a normal glucose phenotype in several of our gin mutants, that demonstrates the importance of this hormone in the glucose responses. In contrast this phenotypic reversion is not observed in the gin6 mutant, suggesting that this gene act downstream of ABA. The expression of several genes regulated by glucose has been analysed in the *gin6* mutant under different conditions. We have also observed an important developmental regulation of the glucose-mediated expression of *ABI4* . Interestingly, ABI4 has been also implicated in osmotic and salt stress responses; thus the possible interaction with sugar regulation is discussed. Further physiological and molecular characterization of gin6 and other gin mutants will also be presented.

Arenas, et al. 2000. Genes Dev. 14: 2085-2096.
Finkelstein et al.. 1998. Plant Cell.10(6):1043-54.

127 A Search for Regulators of the Gibberellin Biosynthetic Gene GA1 in Arabidopsis thaliana

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Accumulating data suggest that environmental factors and internal programs regulate plant development by controlling gibberellin (GA) biosynthesis and tissue responsiveness to GAs. Our research interests focus on developmental regulation of the *GA1* gene, which encodes copalyl diphosphate synthase catalyzing the first committed step of Arabidopsis GA biosynthetic pathway. It has been shown that *GA1* gene expression is regulated in a cell-type and tissue-specific manner. A genetic screen was performed using *GA1-GUS* gene fusion as a reporter to identify mutants defective in *trans*-acting factors regulating *GA1* gene expression. From an ethylmethane sulfonate-mutagenized M2 population, putative mutants were isolated based on altered GUS staining pattern at the adult stages. However, reduced GUS staining in several plants was caused by gene sliencing. In addition, the mutants with increased and ectopic GUS activity did not accumulate a higher level of the *GA1* mRNA. One mutant with elevated level of GUS activity in developing seeds displays maternal-effect embryo lethality. We hypothesize that enhanced GUS activity is due to a loss of a negative regulator that normally suppresses the *GA1* expression and that the expression of the endogenous *GA1* gene is tightly controlled by a *cis*-acting element that is not included in the *GA1-GUS* reporter gene. More analyses are currently underway to test this hypothesis.

128 The Effects of Biotin Depletion on the Expression of Biotin-Containing Enzymes

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Abstract

Biotin is an essential water-soluble vitamin required by all living organisms for normal cellular functions and growth. It is biosynthesized by plants, some fungi, and most bacteria. Biotin acts as a small coenzyme that binds covalently to a lysine amino group of carboxylases to facilitate the transfer of CO_2 during carboxylation and decarboxylation reactions.

The reactions catalyzed by these enzymes are involved in diverse metabolic processes including fatty acid biosynthesis, gluconeogenesis, and the catabolism of amino acids. Although the biological functions of biotin have been well recognized, little is known about the role of this cofactor in regulating gene expression, especially in plants. 3-Methycrotonyl-CoA carboxylase (MCCase) is a mitochondrial biotin-containing enzyme whose major metabolic role in plants is the catabolism of leucine. It contains two subunits, the biotinylated, MCC-A subunit and non-biotinylated, MCC-B subunit. As an initial investigation to ascertain whether and how the expression of biotin-containing enzymes are regulated by their prosthetic group, biotin, we performed western blot analysis and compared MCCase expression patterns between wild-type and *bio1* mutant (*bio1* seedlings can not biosynthesize biotin) *Arabidopsis* plants. We also measured the GUS activity mediated by *MCC-A* and *MCC-B* promoters in both wild-type and *bio1* genetic backgrounds. These studies led to the discovery that MCC-A and MCC-B accumulations were down-regulated (directly or indirectly) by biotin at translational or/and post-translational level. Although biotin had no effects on MCCase gene transcripts, *bio1* plants gradually lost their ability in response to dark- or CO_2-free-adaption when biotin was gradually depleted. These results indicate that biotin is required for the metabolic control *MCC-A* and *MCC-B* genes at the transcriptional level.

129 MOLECULAR AND GENETIC STUDIES OF CASTOR BEAN 2S ALBUMIN GENE IN ARABIDOPSIS

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The castor plant has considerable economic value because of its oil-rich seeds. Up to 90% of the fatty acid content of the oil is 12-hydroxy oleate. As a result, castor oil and products derived from it are used for bio-based lubricants, paints and coatings, plastics and anti-fungals. The US requires 110 million pounds of castor oil per year, all of which is imported. The castor plant is not cultivated in the US, because the castor bean from which the oil is obtained contains the potent toxin ricin as well as highly allergenic proteins. The ultimate goal of our project is to use antisense genes to block production of these hazardous components in castor bean by genetic transformation of castor plants. The 2S albumin in castor bean extract has been identified as the major allergen of castor bean (Bashir et al. Int.Arch. Allergy Immunol 115: 73-82; 1998). The genomic DNA for the castor 2S albumin gene has been cloned and sequenced (Irwin & Lord. Nucleic Acids Res 18: 5890; 1990). To construct effective antisense 2S albumin genes, we have isolated the upstream sequence of the 2S albumin gene by PCR-based gene cloning. These putative promoters are fused to a GUS reporter gene, and the constructions introduced into Arabidopsis plant. The regulatory specificity of these promoters is being tested by measuring the GUS activity in transgenic Arabidopsis.

130 *TREHALOSE-6-PHOSPHATE SYNTHASE 1* is essential for Arabidopsis embryo maturation

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Sugars play a pivotal role in plants acting both as carbon currency and as metabolic signals, controlling many aspects of plant growth and development in response to changes in nutritional status. It has recently been discovered that genes encoding enzymes responsible for the biosynthesis of the disaccharide trehalose exist in many higher plants, but that levels of trehalose are generally extremely low. This finding, combined with the observation that over expressing heterologus genes encoding these enzymes leads to changes in morphology and altered metabolism, has fuelled interest in the physiological role of the pathway [Goddijn and van Dun (1999) Trends in Plant Sci. 4, 315-319].

An Arabidopsis trehalose-6-phosphate synthase gene (*TPS1*) encoding the first enzyme of trehalose biosynthesis has previously been isolated by complementation of the *Saccharomyces cerevisiae tps1*Δ mutant [Blazquez et al., (1998) Plant J. 13, 685-690]. Here we report on an Arabidopsis *tps1* mutant. The mutant is recessive and embryo lethal. Embryo morphogenesis is normal but development is retarded and eventually stalls early in the phase of cell expansion and storage reserve accumulation. The data we will present establishes for the first time that trehalose metabolism plays an essential role in plants and implicates the pathway in the regulation of metabolism during embryo maturation.

131 Identification of 4–Coumarate:Coenzyme A Ligase (4CL) Substrate Recognition Domains

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4-coumarate:CoA ligase (4CL), the last enzyme of the general phenylpropanoid pathway, provides precursors for the biosynthesis of a large variety of plant natural products. 4CL catalyzes the formation of CoA thiol esters of 4-coumarate and other hydroxycinnamates in a two step reaction involving the formation of an adenylate intermediate. 4CL shares conserved peptide motifs with diverse adenylate forming enzymes such as: firefly luciferases, non-ribosomal peptide synthetases, and acyl:CoA synthetases. Amino acid residues involved in 4CL catalytic activities have been identified, but domains involved in determining substrate specificity remain unknown. To address this, we took advantage of the difference in substrate usage between the *Arabidopsis thaliana* 4CL isoforms At4CL1 and At4CL2. While both enzymes convert 4-coumarate, only At4CL1 is also capable of converting ferulate. Employing a gene family shuffling approach, we generated various At4CL1/At4CL2 fusion proteins and determined the enzymatic properties of the chimeric proteins. Two adjacent domains involved in substrate recognition were identified and are referred to as substrate binding domain I (sbdI) and substrate binding domain II (sbdII). At4CL1 and At4CL2 differ in nine amino acids within sbd I and four within sbd II, suggesting that these play roles in substrate recognition. Both sbd I and sbd II of At4CL1 alone were sufficient to confer ferulate utilization ability upon chimeric proteins otherwise consisting of At4CL2 sequences. In contrast, sbd I and sbd II of At4CL2 together were required to abolish ferulate utilization in the context of At4CL1. Based on secondary structure predictions sbd I corresponds to a region previously identified as the substrate binding domain of the adenylation subunit of bacterial peptide synthetases, while sbd II centers on a conserved domain of so far unknown function in adenylate forming enzymes (GEI/LxIxG). The mechanism by which residues within sbd II dictate differences in substrate recognition is likely to be indirect. Residues within sbdII may restrict or enhance access of different substrates to the binding pocket or they might interact with amino acid residues in sbd I that form the substrate binding pocket and thereby modulate substrate recognition indirectly.

132 The pentose phosphate/phosphate translocator from *Arabidopsis thaliana*: Transport properties and genomic characterization

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By database search we identified the *Arabidopsis thaliana* EST clone 121I21T7 (accession n° T43612) showing significant homology to the glucose-6-phosphate/phosphate translocator (GPT) of *Pisum sativum*. The full-lenght cDNA encodes a protein of 417 amino acids. The heterologously expressed protein (RPT) was used to study its transport characteristics. The RPT accepts Pi, trioseP and xylulose-5-P as substrates and is in principle capable of transporting ribulose-5-P and erythrose-4-P, but lacks the ability to transport glucose-6-P, the main substrate of the GPT. Thus, the RPT represents a novel, fourth phosphate translocator family. The RPT is encoded by a single-copy gene located on chromosome V. Differential RT-PCR confirmed that the gene contains no introns. RT-PCR analysis revealed ubiquitous expression, while promoter-GUS fusions showed expression in vegetative parts such as leaves at all developmental stages, roots and stems. In the floral tissue, GUS activity was detected in sepals, style and filaments, but not in petals, young ovaries and anthers. The possible physiological role of the RPT is discussed.

[1] These authors contributed equally to this work

133 Natural variation in carbohydrate metabolism and partitioning in Arabidopsis thaliana

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Carbohydrate metabolism and source-sink interactions in *Arabidopsis thaliana* are studied, in order to understand plant performance. Genetic variation as present in natural populations (accessions) is used as a tool to unravel the genetic background of plant performance. Screening of over 100 accessions revealed large variations for: carbohydrate levels (soluble sugars and starch) in leaves of vegetative plants; diurnal patterns of accumulation of carbohydrates in leaves; activity of enzymes involved in primary metabolism; export of assimilates from leaves. Levels of soluble sugars (glucose, fructose and sucrose) in leaves showed a more than 10-fold variation between accessions. Most accessions accumulated starch in the leaves during the light period, but some did not: starch levels could either be constantly low, or remain high. In addition activities of several enzymes, determined in light-grown seedlings, showed a large variation, both in activity and in tissue localisation. Export of assimilates was determined, using the EDTA-exudation technique, revealing a large variation in total phloem sugars. Crosses are being made between several of the extreme accessions and the standard strain Landsberg erecta, in order to produce mapping populations, which will allow QTL mapping of the various traits.

134 A Mutation in a Major LHCII Gene Causes a Defect in Thermal Dissipation in *Chlamydomonas reinhardtii*

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Although light is essential for photosynthetic organisms, exposure to excess light can be damaging. Thermal dissipation of excess absorbed energy in the light-harvesting complex of photosystem II is a short-term photoprotective mechanism. Thermal dissipation minimizes the accumulation of hazardous reactive oxygen species by both diminishing the excitation of Photosystem II and reducing formation of triplet chlorophyll in the light-harvesting complex. We are using *Chlamydomonas reinhardtii* to study thermal dissipation; dissipation can be measured as non-photochemical quenching of chlorophyll fluorescence (NPQ). After insertional mutageneis, several mutants defective in NPQ were isolated by digital video imaging of chlorophyll fluorescence during exposure to high light (Niyogi et al., 1997). One of these mutants, *npq5* was shown to have an insertion in Lhcbm1, a novel gene encoding a major PSII light-harvesting protein. Transformation with wild-type Lhcbm1 complements the *npq5* phenotype. *npq5* is specifically defective in qE, the pH dependent component of NPQ. *npq5* shows wild-type state transition, photosynthesis, growth at moderate light and high-light triggered violaxanthin de-epoxidation. However, LHCII trimer composition is altered in *npq5*. Pigment analysis is consistent with a decrease in LHCII trimers; there is an increase in the Chl *a:b* ratio and a decrease in the both the amount of Chl *b* and neoxanthin per cell. Furthermore, under high-light, *npq5* suffers both greater photodamage and a diminished growth rate compared to wild-type. Whether the defect in thermal dissipation is due to the specific deletion of Lhcbm1 or the general decrease in LHCII trimers is currently being investigated using site-directed mutagenesis.

135 CARBONIC ANHYDRASE IN ARABIDOPSIS: ROLE AND REGULATION OF EXPRESSION

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B-carbonic anhydrase (CA), an abundant foliar enzyme that catalyzes the reversible hydration of CO_2, is presumed to be a component of the photosynthetic carbon assimilation pathway in plants but its role is not yet fully defined. Although direct evidence is lacking, most data suggest that CA is involved in facilitating CO_2 diffusion across cellular membranes and in the provision of CO_2 as a substrate for the primary photosynthetic carboxylase, Rubisco. To better understand the role of CA in photosynthesis, both antisense and over-expression strategies have been used to modify CA expression levels and to examine the impact of these changes on Arabidopsis growth and photosynthetic capacity as well as β-CA isoform and Rubisco expression dynamics. Data show that prior to full expansion of true leaves, low levels of CA in antisense plants impede seedling growth in high light environments, and that this inhibition can be overcome by the provision of sucrose in the medium or by elevated concentrations of CO_2. The photosynthetic capacity of the cotyledons appears to be reduced at this stage of growth. Photosynthesis and growth of older antisense plants with low CA activity is not reduced at ambient levels of CO_2, and growth is restricted only at sub-ambient level of CO_2. Both CA promoter-GUS and -GFP constructs have also been used to determine the effect of CO_2 and carbohydrate availability on patterns and localization of expression.

136 Overexpression of early gibberellin biosynthesis genes

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The plant growth hormone gibberellin (GA) is important for plant growth and development in processes including seed germination, stem elongation and flower and fruit development. Biosynthesis of GA is regulated by both environmental and endogenous factors to produce appropriate hormone levels. In the GA biosynthetic pathway, the precursor geranylgeranyl pyrophosphate is converted to *ent*-kaurene in reactions catalyzed by copalyl pyrophosphate synthase (CPS) and kaurene synthase (KS). These enzymes are encoded by the *GA1* and *GA2* genes of Arabidopsis, respectively. *ent*-Kaurene is converted to bioactive GA through a series of GA-intermediates. Overexpression of a 20-oxidase gene, whose product catalyzes several downstream steps in GA biosynthesis, is sufficient to confer increased levels of bioactive GA and some aspects of GA-overdose morphology in Arabidopsis. We are interested in understanding whether overexpression of *GA1* and *GA2* in the upstream portion of the GA biosynthesis pathway may also affect GA biosynthesis overall. Transgenic plants have been generated to express the *GA1* and / or *GA2* coding regions under the control of a CaMV 35S promoter. We find that GA1 and GA1/GA2 overexpression lines show increased resistance to the GA biosynthesis inhibitor paclobutrazol. However, none of the overexpression lines shows aspects of GA-overdose morphology. GA measurements indicate that the GA2 overexpressor shows no increase in *ent*-kaurene or early GA intermediates, while the GA1 and GA1/GA2 overexpression lines produce high levels of *ent*-kaurene relative to wild type. None of the overexpression lines has an increase in bioactive GAs (GA_1 and GA_4) or GA catabolite. Additional experiments are aimed at understanding how normal levels of bioactive GA may be maintained in plants with high levels of the early intermediate *ent*-kaurene.

137 Natural variation for seed storage lipid characteristics in *Arabidopsis thaliana* .

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The principal objective of this work is to develop an understanding at the molecular level of genetic influences upon seed storage lipid accumulation. Knowledge of lipid levels are important in such crops as the commercially important Brassica species, however, it is very difficult to clone the genes involved directly from Brassica species due to their large and complex genomes. To overcome this, we aim to identify the corresponding genes in *Arabidopsis thaliana*.The initial stage involves screening different accessions for useful variation and to use that information in a genetic mapping approach to identify QTLs contributing to the quality and quantity of seed lipids. Twelve accessions were selected after initial screening of 364 accessions and put forward to generate recombinant inbred lines. It has been observed that seed lipid traits in the *Arabidopsis* accessions are influenced by environmental conditions. To study this further, populations segregating for oil content and desaturation have also been grown in a controlled environment and will be genotyped. It has also been necessary to develop a suitable marker system for these populations. These are predominantly microsatellite markers, assayed by multiplexed gel electrophoresis. Now the marker system is in place, genotyping of the F_2 populations has been initiated the results of which, will be analyzed by the QTL Mapmaker program to produce maps of the traits under study.

138 Nitrate, Sugar, and Light Regulation of Amino Acid Transporter Expression

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In higher plants, amino acids are the currency of nitrogen exchange between sites of primary assimilation and import-dependent tissues. The partitioning of amino acids in this resource allocation process requires the activity of several amino acid transporters in the plasma membrane.In the results reported here, we show that the expression level of one of these transporters is regulated as a function of nutrient status and environmental cues. The transcript of AAP1, a proton-amino acid symporter, in mature leaf tissue is induced by both nitrogen and carbon sources. AAP1 message is highly induced in nitrogen depleted plants after feeding KNO_3 for 30 min. 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. We show that both sucrose or glucose feeding induces AAP1 message in dark- adapted plants, suggesting light induction is an indirect effect of sugar-signaling. However, we can not rule out a role for a photoreceptor and therefore, we are exploring this question directly with defined light treatments and non-responsive mutants. These results demonstrate that AAP1 expression is regulated by key metabolites that tie its expression to the global distribution of organic nutrients.

139 Structural determinants of Ca^{2+} transport in the CAX genes

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Ca^{2+} levels in plants are controlled in part by H^+/Ca^{2+} exchangers. The Arabidopsis H^+/Cation exchangers, CAX1 and CAX2 were identified by their ability to suppress yeast mutants defective in vacuolar Ca^{2+} transport. CAX1 has a much high capacity for Ca^{2+} transport than CAX2, and CAX1 appears to help regulate plant cytosolic Ca^{2+} levels; however, the amino acid residues involved in CAX-mediated Ca^{2+} binding and translocation have not been identified. An Arabidopsis thaliana homolog of CAX1, CAX3, is 77% identical (93% similar) and when expressed in yeast localized to the vacuole but does not suppress yeast mutants defective in vacuolar Ca^{2+} transport. Chimeric constructs and site directed mutagenesis showed that CAX3 can suppress yeast vacuolar Ca^{2+} transport mutants if a nine amino acid region of CAX1 is inserted into CAX3. A single leucine to isoleucine change within this region caused CAX3 to weakly suppress the yeast Ca^{2+} sensitivity. Biochemical analysis in yeast showed that these alterations caused increased vacuolar H^+/Ca^{2+} exchange. This nine-amino acid region is highly variable among the plant CAX-like genes and exchanging the nine-amino acid region of CAX1 into CAX2 greatly increase the vacuolar Ca^{2+} transport properties of this chimeric protein. This study suggests that this region is involved in CAX-mediated Ca^{2+} transport. We are currently attempting similar studies to define regions important for CAX mediated metal transport.

140 Identification and Characterisation of an *Arabidopsis* auxin glucosyltransferase

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A screen of thirty-six putative *Arabidopsis* UDP-glucosyltransferases (UGT) synthesised as recombinant fusion proteins in *E. coli* led to the identification of a single enzyme capable of conjugating the plant hormone auxin. This protein has been biochemically characterised and found to have highest activity towards the auxin indole acetic acid (IAA). The gene encoding this UGT has been shown to be expressed in siliques and at a lower level in roots.

Transgenic over-expressing plants containing the IAA-UGT coding region fused to the constitutive Cauliflower Mosaic Virus *35S* promoter, show a phenotype consistent with reduced auxin levels. The sensitivity of these transgenic plants to exogenous auxin supports the *in vitro* substrate specificity of the enzyme. The role of this IAA-UGT and the IAA-glucose conjugate it produces are the subject of further investigations.

141 Molecular genetic analysis of CYP78A mutants in *Arabidopsis thaliana*

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Cytochrome P450s are involved in the metabolism of most phytohormones including auxin, GA, cytokinin, BR, ABA, and JA as well as many secondary metabolites in plant cells. Sequencing of the Arabidopsis genome revealed that it contains six different CYP78A genes, CYP78A5 to 78A10. Gene expression pattern analysis showed that they are expressed differentially among various tissues. In an effort to elucidate the functional roles of CYP78A genes in *A. thaliana*, we isolated knock-out mutants for these genes. Only *cyp78A5* mutant showed a visible phenotype having slightly reduced apical dominance. To further elucidate function and to circumvent gene redundancy, we generated double mutant lines based on gene expression patterns (cyp78A5/A7) and sequence identity (cyp78A6/A9, cyp78A6/A8 and cyp78A8/A9). A double mutant, *cyp78A5/A7*, showed pleiotropic phenotypes; short hypocotyls, round rosette leaves, short petiole, dwarfism, and sterility. Trasngenic lines overexpressing CYP78A7 showed strong apical dominance and defects in floral development. The defects in floral development were similar to those in transgenic lines overexpressing CYP78A5. These results suggest that CYP78A5 and CYP78A7 may be involved in the same metabolic network. The phenotype of *cyp78A5/A7* suggests involvement in auxin or GA homeostasis. We will discuss the putative functional roles of the CYP78A genes in growth and development in Arabidopsis.

This work was supported by Human Frontier Science Program grant no. RG0280/1999-M and USDA grant no. NRICGP9701472.

142 Biochemical and Genetic Analysis the *HY1* and *HY2* Genes for Phytochrome Chromophore Biosynthesis in Arabidopsis

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Phytochrome can recognize light via tetrapyrrolic chromophore, phytochromobilin (PΦB), attached to apo-phytochrome protein by thioether bond. The Arabidopsis long hypocotyl *hy1* and *hy2* mutants have defects in light perception since PΦB is not synthesized. We have isolated the *HY1* and *HY2* genes by using map-based cloning approach and found that they encode heme oxygenase and PΦB synthase, respectively. Heme oxygenase catalyzes ring breakage of heme to biliverdin (BV) with concomitant release of carbon monooxide, and PΦB synthase catalyzes reduction of BV to PΦB. Both enzymes were localized in plastids and their activities were depending on reduced ferredoxin. By exploiting the sequence of *HY2*, genes encoding bilin reductase for the biosynthesis of the pycobiliprotein chromophore were identified from oxygenic photosynthetic organism. Moreover, molecular identification of *HY1* and *HY2* finally enabled us to analyze genetic and biochemical regulation of whole tetrapyrrole biosynthetic pathway for chlorophyll, heme, and PΦB in plants. Diurnal expression of genes seemed to be important for the metabolic flux of the pathways. We would like to show recent understanding of the coordinated regulation in tetrapyrrole biosynthetic pathways in plants, in particular, focused on linkage between phytochrome chromophore biosynthesis and photomorphogenesis in plants.

143 Sugar sensing mutants in Arabidopsis

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How plants perceive sugars and react to changes in their cellular sugar status is still largely unknown. It is becoming clear that sugar mediated signals are integrated with other pathways such as light-, and hormone signalling pathways. We isolated Arabidopsis mutants that showed abberant responses to sugars when germinating on various sugars. Mutants that were able to germinate on low amounts of mannose were named mig (mannose insensitive germination) mutants. Mutants that were able to develop and grow on a high concentration of sucrose in the medium were named sig (sucrose insensitive growth). Mutants not able to germinate on high amounts of sucrose were named sss (sucrose super sensitive). Similar phenotypes were found on high amounts of glucose. Further analysis of several of the mutants showed that the expression of carbohydrate regulated genes like rbcS (small subunit of Rubisco) and/or AGPase (ADP-glucose pyrophosphorylase)was altered. The mutants were crossed back to the wildtype and the progeny was analysed. For some of the mutants linkage between the sugar-sensing phenotype with a transposon insertion could be seen. Cloning of the flanking DNA revealed the identity of the gene(s) involved. Further genetic and physiological analysis of the mutants will be discussed.

144 Investigation of a novel group of plant hexokinases

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Plant hexokinases not only play an important role in plant metabolism, but are also thought to be crucial part of the complex plant sugar sensing system (Sheen et al., 1999). The molecular nature of all hexokinase-activities that have been characterized at the biochemical and physiological levels is not yet fully understood. The number of cDNAs isolated from different plant species encoding putative hexokinases is rapidly increasing. Most plant hexokinases published in the literature and in sequence databases carry an N-terminal membrane anchor, as it was first described for spinach hexokinase 1 (SoHxK1; Wiese et. al., 1999). For SoHxK1, we demonstrated that the N-terminal membrane anchor is required for binding of SoHxK1 to the chloroplast outer envelope membrane. Using a low-stringency hybridization screening of cDNA libraries from spinach and potato, we identified a new family of hexokinases that does not possess a membrane anchor. The tissue specific expression of these hexokinases was investigated by RNA gel blot analysis. To study the in vivo function of these hexokinases, we expressed the endogenous gene in potato under the control of the 35S-CaMV-Promotor in sense and antisense orientations. The resulting transgenic plants with increased and reduced expression of that novel potato-hexokinase (StHxK2) will be investigated on their possible changes in metabolism as well as in their possible changes in sugar sensitivity. In addition, we tested the effects of overexpression of HxK2-type hexokinases in *Arabidopsis th.* and yeast (see accompanying poster by Wiese et al.).

Sheen et al., 1999; Curr. Opin. Plant Biol. 2, 410 - 418; Wiese et. al., 1999; FEBS Lett. 461, 13 - 18

145 Characterisation of the arabidopsis mutant *ROOT MERISTEMLESS1*

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Glutathione (GSH, gamma-glutamylcysteinyl glycine) is a ubiquitous low molecular weight thiol in plant cells which is important in the development of the root meristem. GSH is synthesised in a two step pathway from its constituent amino acids by two enzymes, gamma-glutamylcysteine synthetase (GCS) and glutathione synthetase. Previously the *ROOT MERISTEMLESS1* mutant (*rml1*) has been described (Vernoux et al., 2000). *rml1* has a mutation in the GCS gene and consequently has less than 1% of wild-type levels of GSH. The GSH deficiency in *rml1* causes a distinct root phenotype; *rml1* root meristem cells are arrested in cell division at the G1 checkpoint resulting in no postembryonic development of the root meristem. In this study we investigated the *rml1* phenotype using genetic, molecular genetic and biochemical techniques. Although the root meristem does not undergo cell divisions the shoot meristem continues to develop producing a stalk and floral structures. In the presence of GSH the root phenotype is rescued however *rml1* is sterile and exhibits a loss of apical dominance appearing bushy. Preliminary GSH data has shown that in both leaves and roots there is a comparable depletion of GSH. This strongly suggests that GSH has a specific role in the mediation of cell cycle within the root mersitem. Presently we are investigating the reduced and oxidised pools of glutathione within these tissues. The role of GSH in cell cycle mediation is being studied by an examination of the expression of the GCS gene and testing the suppression of the *rml1* phenotype by overexpressing a D-type cyclin. In conjunction with these experiments we are performing kinase assays for Atcdc2a activity.

Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S, Brown S, Maughan SC, Cobbett CS, Van Montagu M, Inze D, May MJ, Sung ZR. (2000) The ROOT MERISTEMLESS1/CADMIUM SENSITIVE2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *Plant Cell*. 12(1): 97-110.

146 A fluoroorotic acid-resistant mutant of Arabidopsis specifically defective in the uptake of pyrimidine bases

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We have previously isolated a fluoroorotic acid (FOA)-resistant mutant GM302 of *Arabidopsis thaliana* that was due to a single nuclear recessive gene *for1* (Mourad and Snook, 1997). Uridine monophosphate synthase (de novo synthesis) and thymidine kinase (salvage synthesis) assays confirmed that FOA resistance in *for1/for1* was not due to amplification of either of these two enzymes. Using ^{3}H-labeled pyrimidine bases, pyrimidine nucleosides, purine bases and purine nucleosides, *for1/for1* plants were defective in the uptake of pyrimidine bases only. Uptake kinetic studies revealed that *for1/for1* plants had decreased affinity for ^{14}C-FOA compared to wild type. We crossed our *for1/for1* mutant with the previously isolated *fur1/fur1* mutant (Wu and King, 1994) known to be specifically defective in the uptake of pyrimidine nucleosides. Testing the F1 progeny, we found that *for1* and *fur1* are two mutant alleles that belong to two different genetic loci, FOR1 and FUR1 respectively. In addition, we have transformed our *for1/for1* mutant using the uracil transporter gene *uraA* of *E. coli* which was PCR-amplified and then fused to the CaMV 35S promoter (p35S-*uraA*). Transformants were complemented and exhibited sensitivity to FOA when included in the growth medium. The above results strongly suggest that the for1 mutant allele affects a transporter system that is specific for the uptake of pyrimidine bases. To our knowledge, GM302 (*for1/for1*) is the first whole plant mutant defective in the uptake of pyrimidine bases.

147 Autoregulation of mRNA Stability of Cystathionine γ-Synthase (CGS), the Key Enzyme for Methionine Biosynthesis

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CGS catalyzes the first committed step of Met biosynthesis. Studies with Arabidopsis *mto1* mutants that over-accumulate soluble Met revealed that the stability of CGS mRNA is downregulated in response to Met application and that the *mto1* mutation abolishes this regulation. Five independently isolated *mto1* mutants carried single-base changes within the coding region of the first exon of CGS, giving rise to amino acid sequence alterations. Transfection experiments suggested that CGS exon 1 coding region is involved in this regulation and in vitro mutagenesis indicated that it is the amino acid sequence that has a role in this regulation. Furthermore, co-transfection experiments using two reporter plasmids suggested that the CGS exon 1 acts in cis. Based on these observations we have proposed a model that this regulation occurs during translation when the nascent polypeptide of CGS exon 1 is in close proximity to its own mRNA (Chiba et al. Science 286: 1371-4 1999).

Since the transfection experiments were carried out by studying reporter activities, evidence to show that the exon 1 coding region of CGS directs the downregulation at the level of mRNA was needed. To test this, transgenic Arabidopsis harboring a chimeric gene in which CGS exon 1 coding region was fused in-frame with GUS or GFP reporter gene and placed under the control of CaMV 35S promoter were constructed. Northern blot analyses indicated that the exon 1 coding region in fact is necessary and sufficient for downregulating the mRNA accumulation in response to Met application.

In order to determine the functional region within the exon 1 of CGS, we made sequential deletions of the exon 1. Transfection experiments indicated that a 41 amino acid region of exon 1 is necessary for the downregulation, although the response was weaker than the full-length exon 1. Alignment of CGS amino acid sequences from 7 plant species shows that this region is highly conserved whereas the overall homology of the exon 1 region is low. Alanine-scanning experiments further narrowed down the necessary region to <14 amino acids (aa 76-88), where all the *mto1* mutations were found to be clustered.

148 Plastid redox state and sugars – interactive regulators of nuclear encoded photosynthetic gene expression

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Feedback regulation of photosynthesis by carbon metabolites has long been recognised but the underlying cellular mechanisms that control this process remain unclear. Using an *Arabidopsis* cell culture we have recently shown that a block in photosynthetic electron flux prevents the increase in transcript levels of chlorophyll a/b binding protein (*CAB*) and the small subunit of Rubisco (*RBCS*) that typically occurs when intracellular sugar levels are depleted (Oswald et al., 2001, *PNAS* Vol 98: 2047-2052. In contrast the expression of the nitrate reductase (*NR*) gene, which is induced by sugars, is not affected. These findings were confirmed *in planta* using Arabidopsis carrying the firefly luciferase reporter gene (*LUC*) fused to the plastocyanin (*PC*) and *CAB2*photosynthetic gene promoters. Transcription from both promoters increases upon carbohydrate depletion. Blocking photosynthetic electron transport with 3-(3',4'-dichlorophenyl)-1,1'-dimethylurea (DCMU) prevents this increase in transcription. We conclude that plastid derived redox signalling can over-ride the sugar regulated expression of nuclear encoded photosynthetic genes. In the sugar response mutant *sun6* (sucrose uncoupled) *PC-LUC* transcription actually increases in response to exogenous sucrose rather than decreasing as in the wild type. Interestingly, plastid derived redox signals do not influence this defective pattern of sugar regulated gene expression in the *sun6* mutant. A model, which invokes a positive inducer originating from the photosynthetic electron transport chain, is proposed to explain the nature of the plastid-derived signal.

149 The effect of phytohormones on storage reserve mobilisation during germination in Arabidopsis

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During germination and early post-germinative growth, seed storage reserves are broken down to provide energy that is required for seedling establishment. Although the regulatory mechanisms controlling germination remain unclear it is known that in many species the phytohormone Abscisic Acid (ABA) has a role in inhibiting germination whilst Gibberellic Acid (GA) has a role in promoting germination. In cereals it has been shown that ABA and GA can regulate the expression of genes involved in the mobilisation of carbohydrate and protein storage reserves during germination. It has been suggested that the inhibitory effects of ABA on the germination of Arabidopsis seeds may be due to inhibition of storage reserve breakdown, which would prevent the embryo from obtaining sufficient sugars for growth. We have investigated the role of ABA in the control of storage lipid and protein breakdown in germinating Arabidopsis. We demonstrate that whilst ABA treatment does not inhibit the expression of genes involved in lipid breakdown, it does result in significant metabolic changes, even in ABA insensitive mutants.

150 Dicarboxylate Transport of the Plastidial Membrane

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Assimilation of ammonia resulting from nitrate reduction and from photorespiration depends on the operation of the plastidic GS/GOGAT cycle. The precursor for ammonia assimilation, 2-oxoglutarate, is imported into the plastid by a 2-oxoglutarate/malate translocator (DiT1). In turn, the product of ammonia assimilation, glutamate, is exported to the cytosol by a glutamate/malate translocator (DiT2). The import of the precursor for ammonia assimilation and the export of its product is essential for plant metabolism. A mutant in a plastidic dicarboxylate translocator was shown to be vital only under elevated CO_2 partial pressure (Somerville & Ogren, 1983). We have cloned and functionally expressed both DiT1 and DiT2 in yeast cells and will present a detailed analysis of the kinetic properties of the recombinant proteins. The *Arabidopsis* genome contains three genes, all located on chromosome 5, that show significant homology to spinach DiT1 (Weber et al., 1995). The expression patterns of two of the three *Arabidopsis* DiT-homologs were analyzed in transgenic plants harbouring promoter-repoter gene fusions. Results of this analysis will be presented.

Somerville, S.C. & Ogren, W.L., (1983) PNAS *80*, 1290-1294; Weber, A., Menzlaff, E., Arbinger, B., Gutensohn, M., Eckerskorn, C. & Flügge, U.I., (1995) Biochemistry *34*, 2621-2627

151 Positional Cloning of a Gene Encoding a Serine Hydroxymethyltransferase Involved in the Photorespiratory Pathway

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Serine hydroxymethyltransferase (SHMT) catalyzes the reversible conversion of serine and tetrahydrofolate (THF) to glycine and 5,10-methylene THF. In the plant photorespiratory pathway, the recycling of 2-phosphoglycolate formed by the oxygenating activity of RubisCO involves the action of SHMT during decarboxylation of glycine in the mitochondrial matrix by glycine decarboxylase. SHMT transfers the CO_2 resulting from glycine decarboxylation to a second molecule of Gly, thereby forming one molecule of Ser. Using a positional cloning approach, we have mapped the defective gene in a photorespiratory *Arabidopsis* mutant and sequenced the corresponding mutation. The defective gene maps to 95 cM on chromosome 4. Using CAPS-markers, we could locate the gene defect on BAC F20D10 that contains a gene encoding a SHMT. Complementation of the mutant by the wild type gene is currently under way. Somerville and Ogren (1981) have isolated and characterized an *Arabidopsis* mutant deficient in SHMT and have demonstrated that this mutant is only viable under elevated CO_2 partial pressure (non-photorespiratory conditions). The *Arabidopsis* genome contains five genes (SHM1 to SHM5). Recently, McClung et al. (2000) identified a candidate gene encoding the SHMT involved in photorespiration (SHM1). The map-based genetic approach also led us to SHM1. The redundancy of SHMT genes obviously is not able to compensate for a loss of function in SHM1, indicating specific roles for SHM2 to SHM5 different from photorespiration.

McClung, C.R., Hsu, M., Painter, J.E., Gagne, J.M., Karlsberg, S.D., Salomé, P.A. (2000) Plant Physiol. *123*:381-391; Somerville, C.R. & Ogren, W.L. (1981) Plant Physiol. *67*:666-671.

152 Genetic and Structural Analyses on the Arabidopsis Glyoxalase II Family of Enzymes

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Glyoxalase II is part of the glutathione-dependent glyoxalase detoxification system. In addition to its role in the detoxification of cytotoxic 2-oxo-aldehydes, specifically methylglyoxal, it has been suggested that the glyoxalase system may also play a role in controlling cell differentiation and proliferation. Because of its role in chemical detoxification, glyoxalase II has been studied as a potential anti-cancer and/or anti-protozoal target; however, very little is known about the active site and reaction mechanism of this important enzyme or the exact role(s) of the enzymes in the cell.

We have previously demonstrated that Arabidopsis contains five putative isozymes for glyoxalase II. In order to better understand the role of the glyoxalase II isozymes in plants we have initiated reverse genetic studies to isolate mutations in the Arabidopsis *GLX2* genes. In addition we have overproduced several of the isozymes and initiated detailed structure/function studies on the enzymes. Data on the analysis of Arabidopsis *GLX2-2* and *GLX2-3* will be presented, including the analysis of knockout mutations for the two genes and structural studies on the two proteins.

153 THE LIGHT CONTROLLED REGULATION OF XANTHOPHYLL COMPOSITION IN LEAVES

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Carotenoids composition in leaves is quite conserved among different higher plants. The predominant carotenoid species are: β-carotene (15-30% of total carotenoids)and the xanthophylls lutein (40-60%), violaxanthin, antheraxanthin, zeaxanthin and neoxanthin (20-40%). Biosynthesis of carotenoids in leaves is light (i.e. phytochrome) independent. However, their composition in chloroplasts is influenced by the intensity of light to which the leaf is exposed. Under strong light the amount of total xanthophylls increases and the ratio between lutein (L) and the xanthophyll-cycle components, zeaxanthin, antheraxanthin and violaxanthin (Z+A+V),decreases. Conversely, the ratio L:(Z+A+V) increases in low light. Lycopene cyclization is a branching point in the carotenoid biosynthesis pathway. One branch, leading to α-carotene and lutein, is catalyzed by the ε-cyclase, whereas the second branch, leading to β-carotene and the other xanthopylls, is catalyzed by lycopene β-cyclase. We have measured the level of expression of the genes *Lcy-b* and *Lcy-e*, encoding lycopene β-cyclase and lycopene ε-cyclase, respectively, in arabidopsis and tomato leaves under different light intensities. In both plant species the ratio of mRNA levels between *Lcy-b* and *Lcy-e* increased five folds under strong illumination relative to low light.Using a gene-silencing technique we have inactivated the expression of lycopene ε-cyclase in tomato plants. No apparent phenotype was observed in these plants in spite of the fact that they lacked lutein. This result indicates that xanthophyll composition in the light-harvesting complexes can be modulated by the carotenoid biosynthesis flux. The rate of non-photochemical quenching of chlorophyll fluorescence (a measure of the protective contribution of xanthophylls) was higher in the lutein deficient mutants. These findings indicate that cyclization of lycopene is a key regulatory step of xanthophyll composition in mature leaves, which contribute to the adaptation of plants to varying light intensities.

154 Sinapoylglucose:choline sinapoyltransferase is a serine carboxypeptidase-like protein that functions as an acyltransferase in plant secondary metabolism

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Serine carboxypeptidase-like (SCPL) proteins have traditionally been assigned roles in the hydrolytic processing of proteins; however, several SCPL proteins have recently been identified as catalysts in transacylation reactions of plant secondary metabolism. We identified an Arabidopsis mutant, *sng2* (*sinapoylglucose accumulator 2*), that is defective in the synthesis of sinapoylcholine, the major sinapate ester accumulated in seeds of Arabidopsis and some other members of the Brassicaceae. The cloning of the *SNG2* gene by a combination of map-based and candidate gene approaches and the expression of *SNG2* in *Escherichia coli* demonstrated that it encodes sinapoylglucose:choline sinapoyltransferase (SCT). Although SCT catalyzes a transesterification reaction, its homology to SCPL proteins places it in a growing class of SCPL proteins that function as acyltransferases in plant secondary metabolism. The mechanism by which this class of SCPL proteins catalyzes acyltransferase reactions is unknown. Classical serine carboxypeptidases employ a catalytic triad of serine, aspartic acid, and histidine residues for catalysis. The deduced amino acid sequence of SCT, as well as the other SCPL proteins with known acyltransferase function, shares all three, conserved, catalytic residues. Because SCT primarily forms inclusion bodies that must be denatured and refolded to generate active protein when expressed in *E. coli*, we have expressed SCT in *Saccharomyces cerevisiae* in order to further characterize the protein and its catalytic mechanism. The *S. cerevisiae vpl1* (*vacuolar protein localization 1*) mutant, which has been shown to secrete yeast Carboxypeptidase Y, was used for expression of SCT. In the *vpl1* mutant background, approximately 97% of SCT activity is found in the media. Analysis of SCT expressed in this system will allow for the characterization of the kinetic parameters of SCT, as well as providing a heterologous system for analyzing the activity of SCT with site-directed mutations of the proposed catalytic residues. In addition, transformation of the site-directed mutants into the *sng2* mutant will also provide for an *in vivo* analysis of the importance of these proposed catalytic residues.

155 Characterization of Lignin-Deficient Mutants

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Lignin is a cell wall polymer formed from the aromatic products of the phenylpropanoid biosynthetic pathway. Lignin is believed to confer both strength and waterproofing to cell walls, and therefore its acquisition is considered to be one of the pivotal evolutionary events during plant colonization of land. One of the most heavily lignified cell types in Arabidopsis is the xylem tracheary element. These cell types are believed to benefit from lignification as waterproofing should assist in efficient water movement, and strength is required to cope with the strong negative pressures associated with the water movement. These functions for lignin, however, are largely based on its position of deposition and its chemical properties. To more directly assess lignin function, we are studying two allelic mutants that fail to accumulate detectable amounts of lignin. We have identified the lesion using a combined biochemical rescue and molecular approaches, and we have characterized the anatomical repercussions of lignin loss using both light and electron microscopy. These experiments show that the loss of lignin results in severe cell wall defects, with tracheary elements crushing almost immediately after the loss of cellular contents (a normal part of the tracheary element developmental program). Prolonged growth of the mutant results in a phenotype strongly resembling that of mutants that over-accumulate auxin. One possible interpretation of these observations is that the lesion affects amino acid pools such that auxin biosynthesis is also affected.

156 Genetic and Biochemical Characterization of UDP Sugar 4-epimerases in *Arabidopsis thaliana*.

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UDP sugars serve as precursors for the biosynthesis of cell wall polysaccharides. Most UDP sugar interconversions involve a reversible 4-epimerization leading to pairs of UDP sugars such as UDP-D-glucose / UDP-D-galactose, UDP-D-glucuronic acid / UDP-D-galacturonic acid, and UDP-D-xylose / UDP- L-arabinose. A UDP-D-glucose 4-epimerase gene (*UGE1*) has been cloned and shown to catalyze the interconversion between UDP-D-glucose and UDP-D-galactose (Dörmann and Benning. Arch. Biochem. Biophys. 1996; 327: 27-34). In order to isolate other genes involved in UDP sugar 4-epimerization including UDP-D-glucuronic acid and UDP-D-xylose 4-epimerases, we screened a cDNA library with expressed sequence tags (ESTs) that showed homology to *UGE1*. This led to the isolation of full-length cDNAs designated *UGE2* and *UGE3*. Additional candidate genes for *UGEs* (*UGE4* and *UGE5*) are predicted from the *Arabidopsis* genome sequence. A coding region for UDP-D-xylose 4-epimerase (*UXE1*) was identified by positionally cloning the gene deficient in the *Arabidopsis* cell wall mutant *mur4*. Recent database searches indicate that the *Arabidopsis* genome contains three sequences with high degree of sequence similarity to *UXE1* (=*MUR4*). Northern analysis showed that *UGE2* and *UXE1* were most highly expressed in stems and flowers. The *UGE2* and *UGE3* cDNAs were expressed in *E. coli* leading to the synthesis of 38kDa proteins displaying both UDP-D-glucose and UDP-D-xylose 4-epimerase activities *in vitro*, a substrate specificity also observed for the UGE1 protein. The *UXE1* cDNA encodes a 40kDa membrane protein, which on expression in yeast (*S. cerevisiae*) displayed UDP-D-xylose 4-epimerase activity. However, neither the *UGE* nor the *UXE1* gene products acted on UDP-D-glucuronic acid *in vitro*. We hypothesize that the *UGE* gene family encodes cytosolic UDP-D-glucose 4-epimerases while the *UXE* gene family encodes membrane-bound UDP-D-xylose 4-epimerases, possibly localized to the Golgi.

Supported by the U.S. Department of Energy (grant No. DE-FG02-95ER20203).

157 Comparative analysis of starch metabolism in starch deficient and starch excess mutants of *Arabidopsis thaliana*

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Loss of function mutants have long been used to analyze starch biosynthesis and degradation pathways in *Arabidopsis*. Mutants in starch biosynthesis (starch-free mutants) include ADP-glucose pyrophosphorylase (*adg1*; Lin et al. 1988), plastidic phosphoglucoisomerase (*pgi1*; Yu et al. 2000) and plastidic phosphoglucomutase (*stf1*; Caspar et al. 1985; Kofler et al. 2000). Starch excess mutant *sex1* (Caspar et al. 1991) is deficient in an *Arabidopsis* homologue of the potato R1 protein (unpublished data) and *sex4* is deficient in a plastidic endo-amylase (Zeeman et al. 1998).

We report here a comparative analysis of these mutants grown under identical conditions. In addition, we report maltose levels in these mutants because we hypothesize that starch conversion to sucrose involves maltose. We used a novel enzymatic system (Shirokane et al. 2000) to determine maltose levels, in additions to the levels of glucose, fructose, sucrose, and starch in leaf extracts of the *Arabidopsis* mutants *sex1-5*, *pgi1*, *stf1*, the corresponding wild types, and in an *stf1*-line that was complemented with the wild type gene.

Throughout a 10-hour light period, maltose was found to be very low in all tested plant lines. During the following night period, degradation of starch in the wild types was accompanied by an increase in maltose levels to about 0.5 µmol/g FW. No maltose was detectable in starch-free mutants throughout a diurnal cycle. We established a clear correlation between starch degradation and the occurrence of maltose in *Arabidopsis* leaf extracts, corroborating our hypothesis that maltose plays an important role as intermediary metabolite in the conversion of transitory starch to sucrose.

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158 Plant hexokinases - subcellular localization and function

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Plant life is dependent on the availability of sugars for respiration and growth. This metabolic function of sugars is complemented by elaborate systems that sense the availability of sugars and initiate a variety of cellular and organism responses. The most notably response is the control of gene expression: a variety of genes are either repressed or induced by sugars (Koch, 1996). Hexokinases catalyze the phosphorylation of hexoses thereby activating them for catabolism or anabolism. In analogy to the yeast glucose-sensing system, plant hexokinases are also thought to act as sugar sensors (Jang et al., 1997). We isolated several hexokinase cDNAs from spinach, tobacco and potato. Comparison of their amino acid sequences revealed that they can be separated into two groups. One of these groups is characterized by an N-terminal membrane anchor. For spinach hexokinase 1 (SoHxK1) it was shown that the anchor is crucial for binding of SoHxK1 to the outer chloroplast envelope membrane (Wiese et al., 1999). Specific antisera, directed against members of each group and GFP-fusions helped to clarify the subcellular localization of these hexokinases. The catalytic activity of the encoded proteins was investigated in plant extracts as well as after heterologous expression of the cDNAs in yeast hexokinase knockout mutants. The possible function of the different hexokinases will be discussed in relation to their tissue-specific expression, subcellular localization and their activities. Heterologous overexpression of the group I and II hexokinases in *Arabidopsis* was used to study whether they influence plant sensitivity towards sugar signals similar to the overexpression of endogenous AtHxK1 and AtHxK2 (Jang et al., 1997). We will also investigate the dependency of the possible sensoric function on the subcellular localization. To check the analogy to the yeast system, we tried to complement the sensing function of the yeast enzyme with different plant enzymes and yeast/plant enzyme chimera in the genetic background of a hexokinase-deficient yeast mutant.

Jang et al., 1997; Plant Cell 9, 5 - 19; Koch, 1996; Annu.Rev.Plant Physiol.Plant Mol. Biol, 47, 509 - 540; Wiese et al., 1999; FEBS Lett. 461, 13 - 18

159 The role of F-box protein AtFBL3 in sugar signal transduction in *Arabidopsis thaliana*

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The perception and signal transduction of sugar and ABA signals are important for plant growth and development. Here we show that an F-box protein, AtFBL3, is involved in a converged ABA and sugar signaling network that controls seed germination and seedling development. AtFBL3 shares high sequence similarity with Grr1, a central regulator of glucose signaling in yeast. The SCF^{Grr1} (Skp1, Cdc53, and F-box protein) in yeast acts as a ubiquitin E3 ligase complex that recruits phosphorylated protein substrates to be ubiquitinated and eventually degraded by the 26S proteasome. A conserved protein-protein interaction between Skp1 and F-box proteins has been found in various eukaryotes from yeast to humans. We demonstrate that AtFBL3 interacts with the Arabidopsis Skp1-like proteins ASK1 and ASK2 in the yeast two-hybrid assay. Transgenic Arabidopsis plants ectopically expressing *35S::AtFBL3* exhibited reduced responses to both sugar and ABA signals that negatively regulate seed germination and early seedling development. In addition, the induction of *ADH* and *AtEM6* by ABA and the repression of *CAB* and *PC* by glucose were also diminished in the *35S::AtFBL3* transgenic plants. Finally, genetic analysis showed that the double mutant *35S::AtFBL3 35S::AtHXK1* behaved like the single mutant *35S::AtFBL3* in both sugar and ABA responsive assays, indicating that AtFBL3 acts downstream of AtHXK1 in the AtHXK1-dependent glucose signaling pathway. We propose that AtFBL3 may act as a subunit of SCF^{AtFBL3} in the ubiquitin-mediated degradation of a positive regulator that participates in the converged sugar and ABA signaling network.

160 A key regulator of vesicle trafficking required for root hair morphogenesis

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We have characterized a novel Sec1, KEULE, required for root hair morphogenesis and cytokinesis in Arabidopsis. We have raised a peptide antibody specific to KEULE and show that KEULE is characteristic of a Sec1 protein[1] in that (1) it exists both on and off membranes, (2) it is peripherally associated with membranes,and (3) it is a syntaxin-binding protein.

In addition to a primary defect in the execution of cytokinesis, root hairs in *keule* mutants are stunted and radially swollen. Of a large collection of cytokinesis-defective mutants (F.A., U. Mayer & G. Juergens, unpublished), we have found another mutant line which, like *keule*, is required for both cytokinesis and root hair morphogenesis. As eight other cytokinesis-defective mutants (F.A., U. Mayer & G. Juergens, unpublished), including *knolle*[2], grow long root hairs, this defect appears to be independent of the cytokinesis defect. Both cytokinesis and root hair morphogenesis require the deposition of new cell walls and in both instances this occurs via polarized secretion.

Sec1 proteins are key regulators of vesicle trafficking, capable of integrating a large number of intra and/or intercellular signals and of transducing such signals to the vesicle fusion apparatus by virtue of a direct interaction with syntaxins on target membranes. By analogy, we postulate that KEULE integrates the developmental, hormonal and environmental signals that regulate polarized secretion giving rise to root hair growth.

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161 Xyloglucan Endotransglycosylases of Arabidopsis: Diversity of Genes, Expression, Regulation and Function

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The plant cell wall is a complex structure composed in part of cellulose microfibrils interconnected by a network of polysaccharides, such as xyloglucan. *In vitro*, xyloglucan endotransglycosylases (XETs) cleave xyloglucan polymers and religate the newly generated reducing end to other xyloglucan polymers. Although the biochemical activity of XETs is well defined, the physiological consequences of this activity *in vivo* remain undetermined.

Previous work has shown that *TCH4* of Arabidopsis encodes an XET, thus TCH4 has the potental to modify an important component of the cell wall. Analysis of the Arabidopsis database reveals an extensive gene family that encodes 30 XET-related (XTR) proteins. The predicted proteins share between 37% and 85% identity as well as a conserved motif (DEIDFEFLG) that is identical to the active site of *Bacillus* β-glucanases and may be necessary for enzymatic function of the XETs.

We are investigating the functional significance of this gene family through the characterization of primary protein structures, enzymatic activities, consequences of loss of function and regulation of gene expression. This work is supported by the NSF (9982654).

162 Kinetic Analysis of Ethylene's Effects on Growth in Etiolated Arabidopsis Seedlings

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Responses to the plant hormone ethylene are mediated by a family of 5 receptors in Arabidopsis that act in the absence of ethylene as negative regulators of response pathways. Ethylene binding is thought to act by suppressing receptor output. We have been using the etiolated seedling growth response to study the dynamics of receptor function. Using a digital infrared camera, the kinetics of hypocotyl growth rate changes resulting from the application and subsequent withdrawal of ethylene were monitored. Application of 10 ppm ethylene led to a drop in growth rate to a new steady-state-rate after 45 minutes. Subsequent removal of applied ethylene after 2 hours of treatment resulted in a return to the original, higher growth rate within 1.5 hours. This relatively fast recovery rate must be reconciled with the much slower rate of ethylene release observed with the yeast expressed ETR1 receptor protein (half life > 11 hours). We are testing the hypothesis that the rapid recovery rate is determined by new receptor synthesis. Upon ethylene withdrawal, new receptors would be synthesized in the unbound, active state, resulting in the suppression of response pathways. Based on this model, we predicted that recovery rate would be slower under conditions in which receptor synthesis rate was decreased. As predicted, we found slower recovery rates after shorter treatment times that precluded induction of the ethylene-inducible receptor isoforms. The recovery rate following longer ethylene treatment was also slower in the etr 2-3 receptor null line. Finally, rates of recovery were particularly slow after all treatment times in the etr 1-7 receptor null line. These results are consistent with our model of receptor function and highlight the involvement of specific receptor isoforms in mediating recovery from ethylene responses. Experiments looking at mRNA levels for each receptor isoform during the response and recovery phases are currently underway.

163 Identification of an Arabidopsis CaaX protease reveals a subcellular targeting pathway for prenylated proteins

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Following prenylation, proteins undergo two prenyl-dependent modifications at their carboxyl-terminal end. First, the aaX moiety of the CaaX box is removed. Then, the free carboxyl group of the prenyl cysteine is methylated. In *Saccharomyces cerevisiae*, CaaX box proteolysis is catalyzed by either of two ER membrane localized, and unrelated proteases called AFC1 and RCE1. Methylation is catalyzed by a prenyl-dependent carboxy methyl transferase (PCM) encoded by the *STE14* gene. Therefore, the subcellular localization of AFC1, RCE1 and PCM, provide a trafficking pathway for prenylated proteins. *AtAFC1*, the Arabidopsis homologue of yeast AFC1 was cloned by PCR from a cDNA library using primers that were designed according to a sequence published by the Arabidopsis genome initiative. Functional complementation of mutant *rce1 Δ afc1 Δ* yeast cells was demonstrated by growth inhibition, pheromone diffusion halo assays. Halos were formed when *AtAFC1* was over-expressed from either a 2 μ high copy or a *CEN* low copy number plasmids. Furthermore, AtAFC1 promoted plasma membrane association of the Arabidopsis GFP-ROP AtRAC7 fusion protein in *rce1 Δ afc1 Δ* yeast cells providing evidence that AtAFC1 can process prenylated plant proteins. A GFP-AtAFC1 fusion protein was localized in the ER following its transient expression in *Necotiana benthamiana*, Arabidopsis and onion epidermal cells. A GFP-AtPCM fusion protein was, however, localized in the reticulate golgi when transiently expressed in the same cells. These data provide evidence that prenylated proteins in plants, are first targeted to the ER and following proteolysis are transferred to the golgi where they get methylated and targeted to their final destination in the cell. Thus, targeting of prenylated proteins may occur through the endomembrane system.

164 Loss-of-function mutations in the ethylene receptor *ETR1* cause enhanced responsiveness to ethylene in Arabidopsis

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Ethylene signal transduction in Arabidopsis proceeds through a linear pathway headed by a family of 5 ethylene receptors that regulate the activity of the downstream MAP kinase kinase kinase, CTR1. Loss-of-function mutants have been generated for 4 of the receptors and these mutations, either as single nulls or in combination, have been examined for their effects on ethylene signaling. Triple- and quadruple-null mutants display an ethylene-response phenotype independent of ethylene perception, indicating that the ethylene receptors function as negative regulators in this pathway. This is presumably due to the loss of activators for CTR1, which is responsible for actively repressing ethylene responses. No ethylene-related phenotype has been described for single-null mutants of the ethylene receptors although it was reported that *etr1* loss-of-function mutants (e.g. *etr1-7*) display a growth defect that limits plant size. We have found that this apparent growth defect actually results from enhanced responsiveness to ethylene in these mutants. This increased ethylene sensitivity is found in all tissues tested including leaves, along with roots and hypocotyls of dark grown seedlings. Additionally, *etr1-7* displays increased resistance to high glucose treatment, a phenotype that has been associated with ethylene perception. The increased sensitivity phenotype can be rescued by the *ein2* loss-of-function mutation, which blocks ethylene perception, indicating that the *etr1-7* phenotype is ethylene dependent. This phenotype does not result from ethylene overproduction, as *etr1-7* seedlings produce levels of ethylene comparable to wild type. Null mutations in 3 other ethylene receptors (*ETR2*, *EIN4*, and *ERS2*) do not cause the observed increase in ethylene responsiveness. RT-PCR shows that this is not due to increased expression of other ethylene receptors that may compensate for the loss-of-function lesions. The unique phenotype associated with loss-of-function mutations in *ETR1* strongly suggests that *ETR1* has an added role in ethylene signaling that is not mediated by the other members of the ethylene receptor family. Possible scenarios relating to the nature of this unique role will be presented.

165 Regulation of G1/S cell cycle transition and initiation of DNA replication

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Transition from G1 to S-phase is one of the key stages where cells regulate progression through the cell cycle. In animal cells, the retinoblastoma (RB) pathway plays a crucial role at this stage. Retinoblastoma-related (RBR) proteins and other components of the RBR pathway, such as the E2F/DP transcription factos, and multiple CDKs and cyclins exist in plants. In Arabidopsis, one RBR gene, six E2F-related and two DP-related genes have been identified in its genome. We want to understand the function of this pathway in regulating cell cycle and genome replication, and its potential role in development. S-phase initiates once CDK/cyclin activity relieves E2F/DP transcription factors from repression. This is believed to be a consequence of specific phosphorylation events on RBR. Initiation of DNA replication is a tightly regulated process. Studies in yeast have revealed that CDC6 protein plays a crucial role in the activation of a multiprotein structure, the origin recognition complex (ORC) which is bound to DNA replication origins.

We are focusing at studying proteins that regulate initiation of S-phase and DNA replication. Here, we discuss our data on an *A. thaliana* cDNA encoding the AtCDC6 protein, and its possible function during developmentally-regulated DNA replication events, e.g. endoreplication. The *AtCDC6* gene is maximally expressed in early S-phase. Consistent with this cell-cycle regulated expression, its promoter contains an E2F consensus site which mediates binding of a plant E2F/DP complex. Transgenic plants carrying an *AtCDC6* promoter-GUS fusion revealed that this promoter is active not only in proliferating cells but also in other locations. Furthermore, AtCDC6 is degraded in a ubiquitin- and proteasome-dependent manner, but with different efficiency in different tissues.

Our studies indicate that (1) *AtCDC6* is expressed in a cell-cycle dependent manner and, most likely, is an E2F target gene, (2) endoreplication seems to require inactivation of the retinoblastoma protein, and (3) development-regulated endoreplication cycles seem to be associated with increased expression of the *AtCDC6* gene and, most likely, stability of AtCDC6.

166 Does AtE2F-I function in cell division and/or cell differentation?

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Cell proliferation is controlled by a diverse group of cell cycle regulators. In animal cells, the retinoblastome protein (RB) plays a crucial role in controlling the transition from G1 to S-phase. In a hypophosphorylated state RB interacts with the transcription factors E2F/DP. This RB/E2F/DP complex has been suggested to function as transcriptional repressor. During the G1-S transition, cyclin-dependent kinases phosphorylate RB, and then E2F/DP factors are liberated, inducing the expression of genes required for cell cycle progression. Recent findings have demonstrated that a RB-related and E2F/DP proteins are also present in plants, suggesting a similar control of cell proliferation in these organisms In *Arabidopsis*, 1 *RRB* , 6 different*E2F-related* and 2 *DP-related* genes have been identified. However, the function of these factors in cell cycle control and differentation programs have not been determined. In this work we have focused on studying the function of one member of these E2F family, *AtE2F-I*. In gel-shift assays we found that the AtE2F-I protein is able to bind, in association with DP, the consensus DNA E2F-binding site. To determine the expression of E2F-I we have analyzed transgenic lines bearing the reporter gene *GUS* fused with the E2F-I promoter. We found that this gene is highly expressed in meristematic zones, along the root system and during flower development. Northern-blot analysis using partially synchronized Arabidopsis cell showed that this gene is expressed in higher levels during the G1-S transition.In order to prevent an excessive or time-deregulated E2F-function, the E2F protein stability is controlled by the ubiquitin pathway. Using an N-terminal region of E2F-I fused to GUS reporter we found that this chimera is degraded in a proteosome dependent manner. Furthermore, we have found an F-box protein, a component of the SCF ubiquitin ligase complex, that interact with E2F-I, suggesting that the stability of this protein is controlled, as some of its human counterparts, through the UBQ-SCF pathway. We are currently generating transgenic plants overexpressing the E2F-I cDNA. These and further results from the E2F-I transgenic lines will be presented at the meeting.

167 RGPs and AMYs: plant protein-self-glycosylation

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In plants, when a total protein extract is incubated with UDP-[3H]Glc for a few minutes, the reaction separated by SDS-PAGE, and the gel dried and exposed to film, a 41-kDa band can be detected. These are the RGPs, the plant Reversibly Glycosylated Polypeptides that have been predicted to be involved in cell wall polysaccharide biosynthesis. Within their protein sequence is domain A, a region shared with the glycosyltransferase 2 (GT-2) family of proteins, and a novel glycosylation site where a single sugar is attached to an arginine residue. The Amylogenins, or AMYs, are proteins that lack domain A but contain the glycosylation site, and in the presence of UDP-[3H]Glc also glycosylate. AMYs are the predicted protein primers for starch biosynthesis. We have taken bioinformatic, molecular biological, biochemical and genetic approaches to unravel the role these proteins have in plant metabolism. Our current results will be presented.

168 Regulation of polar auxin transport by the RCN1 subunit of protein phosphatase 2A

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Polar auxin transport is required for normal growth and development in plants. Several lines of evidence suggest that reversible protein phosphorylation regulates auxin transport, but little is known about the protein kinases and phosphatases involved. Seedlings carrying the *rcn1* mutation exhibit defects in several differential cell elongation responses and show altered growth in the presence of the auxin transport inhibitor, naphthylphthalamic acid (NPA), suggesting altered regulation of auxin transport. The *RCN1* gene encodes an A regulatory subunit of protein phosphatase 2A (PP2A), and *rcn1* mutant seedlings exhibit reduced protein phosphatase 2A activity in vivo and in vitro. Measurement of auxin transport in roots of *rcn1* seedlings shows that reduced phosphatase activity affects both basipetal and acropetal transport streams. Root basipetal transport is increased in *rcn1* or phosphatase inhibitor-treated seedlings, but shows normal sensitivity to NPA. Elevated basipetal transport impedes gravity response, but a normal gravity response can be restored to mutant seedlings by treatment with a low concentration of NPA. Genetic and pharmacological experiments show that elevation of auxin transport does not require the products of the *AGR1*/*EIR1*/*PIN2*/*WAV6* or *AUX1* genes. Root tip morphology appears normal in *rcn1* seedlings, arguing against an anatomical basis for altered basipetal transport. Surprisingly, root acropetal transport is normal in *rcn1* seedlings in the absence of NPA, but shows reduced NPA sensitivity. Lateral root growth also exhibits reduced NPA sensitivity in *rcn1* seedlings, consistent with acropetal transport controlling lateral root growth. These results support the role of protein phosphorylation in regulating polar auxin transport in seedling roots, and suggest that the acropetal and basipetal transport streams are differentially regulated. Additional genetic data suggest that *RCN1* may also play a role in regulating auxin transport during embryogenesis. Activity of the PIN1 auxin efflux carrier is required for development of normal, bilaterally symmetric embryos; *pin1* mutant seedlings exhibit a range of abnormal morphologies. The *rcn1* mutation enhances *pin1* seedling phenotypes, increasing the severity of Pin^{-} phenotypes.

169 Diverse changes in phenylpropanoid metabolism in an Arabidopsis mutant defective in the gene encoding *p*-coumarate 3-hydroxylase

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The end products of the phenylpropanoid pathway play important roles in plant structure, and development, as well as in plants' defenses against biotic and abiotic stresses. From a human perspective, phenylpropanoid pathway-derived metabolites influence both human health and the potential utility of plants in agricultural contexts. The only enzyme of the phenylpropanoid pathway that has not been characterized is *p*-coumarate 3-hydroxylase (C3H). Our lack of knowledge about this enzyme and its corresponding gene represents a critical gap in our understanding of phenylpropanoid metabolism. By screening for plants that fail to accumulate soluble fluorescent phenylpropanoid secondary metabolites, we have identified a number of Arabidopsis mutants that display a *reduced epidermal fluorescence* (*ref*) phenotype. The *ref8* mutant exhibits a red fluorescence under UV light that is similar to the *fah1* mutant, a mutant defective in the ferulate 5-hydroxylase gene. We have cloned the *REF8* gene, and have verified that it encodes C3H by expression of the wild-type gene in yeast. Phenotypic characterization of the *ref8* mutant has revealed that the lack of C3H activity leads to diverse changes in phenylpropanoid metabolism and plant development. First, the *ref8* mutant accumulates *p*-coumarate esters, instead of accumulating sinapoylmalate as found in wild type plants. Second, the mutant deposits a lignin formed primarily from *p*-hydroxyphenyl units, a monomer that is a relatively minor component in the lignin of other plants. Finally, *ref8* mutants are dwarfed and exhibit other abnormalities suggesting that C3H function is required for normal growth and development.

170 Epistasis analyses of *hrl1* reveal novel roles for SA, JA, and Ethylene in signaling leading to defense and cell death in Arabidopsis

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Resistant plants possess an impressive array of defense responses for survival against pathogen attack. In Arabidopsis this defense display is orchestrated by at least three signaling molecules: salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Molecular genetic analyses using mutants that are perturbed in resistance response to pathogens have elucidated multiple signaling pathways that act both synergistically and antagonistically. Epistasis analyses of *hrl1* (*hypersensitive response-like lesions*) with important defense regulators like NPR1, ETR1 and COI1 demonstrate the interactions of signaling pathways that usually act independent of each other. In *hrl1npr1*, JA/ET-dependent *PDF1.2* expression is significantly reduced compared to *hrl1*. This result suggests that NPR1 may have an additional role in regulating *PDF1.2* expression in *hrl1*. In *hrl1nahG* transgenic plants, BTH at very low concentration (1 µM) induces *PDF1.2* but suppresses it at a higher concentration (100 µM). This result clearly suggests that SA at low concentrations may have a synergistic effect in inducing *PDF1.2* and not entirely antagonistic as previously thought. Our experiments with *hrl1etr1* indicate that ethylene-signaling response is necessary for systemic *PR-1* expression and resistance to *P. syringae* in *hrl1*. When JA responses are blocked in *hrl1coi1*, cell death phenotype is exaggerated and the plant is extremely dwarfed suggesting that COI1 might negatively regulate cell death during pathogen infection. These results clearly show that different signaling pathways influence one another and this labyrinth is dependent on the nature and magnitude of the stimuli.

171 ADF proteins are involved in the control of flowering and regulate F-actin organization, cell expansion and organ growth in Arabidopsis

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Mostly based on the results of *in vitro* experiments, ADF (actin depolymerizing factor) proteins are thought to be key modulators of the dynamic organization of the actin cytoskeleton. The few studies concerned with the *in vivo* function of ADF proteins that have been reported to date were almost exclusively performed using single cell systems and have failed to produce consistent results. To be able to investigate ADF functions *in vivo* and during the development of multicellular organs, we have generated transgenic *A. thaliana* plants that express a cDNA encoding an ADF protein (AtADF1) in the sense or antisense orientation under the control of a strong, constitutively active promoter. Selected lines with significantly altered levels of AtADF protein expression were phenotypically characterized. Overexpression of AtADF1 resulted in the disappearance of thick actin cables in different cell types, caused irregular cellular and tissue morphogenesis, and reduced the growth of cells and organs. By contrast, reduced AtADF expression promoted the formation of actin cables, resulted in a delay in flowering and stimulated cell expansion as well as organ growth. These results are consistent with the molecular functions of ADF as predicted based on *in vitro* studies, represent the first set of data on the global roles of ADF proteins during the development of a multicellular organism other than *D. melanogaster* and demonstrate that these proteins are key regulators of F-actin organization, of flowering, as well as of cell and organ expansion in *A. thaliana*.

172 A gene family in *Arabidopsis thaliana* involved in magnesium transport

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The starting point for this work was the identification of two genes that form the yeast magnesium (Mg) uptake system (MacDiarmid and Gardner, 1998). The yeast strain CM52 grows under normal Mg conditions. The deletion of both of the uptake genes from CM52 gives a strain, CM66, which is incapable of growth under normal Mg conditions. A. Tutone used this yeast strain to screen an *Arabidopsis* cDNA library in a yeast expression vector, and isolated a gene (*AtMGT10*) that allowed the mutant yeast to grow at normal Mg concentrations. This gene has significant homology to two bacterial genes that have been shown to be involved in the transport of divalent cations, CorA and MRS2. Nine additional family members have been identified from the public *Arabidopsis* genomic sequence database. A range of tissues, young silique, mature silique, flower, stem, leaf and root were analysed using RT-PCR to test for the expression of each gene. Most of the genes were expressed in every tissue. The exceptions were *AtMGT5*, expressed only in flowers, and *AtMGT8*, which was not detected in stems. RT-PCR was also used to amplify full length copies of each of the nine genes. Each gene was cloned and sequenced to determine the locations of the introns and the protein sequence. The relationships between the genes were analysed. *AtMGT1* and *AtMGT2* are the most closely related genes and *AtMGT7* ? *AtMGT9* form another close grouping. *AtMGT10* is the most divergent of the family members. The neighbour-joining tree will be presented. Each of the nine genes was cloned into a yeast expression vector and screening for transport functions in yeast is currently in progress.

173 Regulation of cell death by LSD1 and it's homologues LOL1 and LOL2

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The LSD1 protein contains three type IV zinc finger domains which share remarkable identity with one another. *LOL1* and *LOL2* (LSD one like), two other Arabidopsis genes were identified, which possess zinc finger domains highly conserved internally as well as to LSD1, indicating that LSD1, LOL1 and LOL2 are members of a small protein family and might have similar function. The *LOL2* gene encodes two proteins, designated LOL2a and LOL2b. LOL2a contains an additional DNA binding motif. During an incompatible interaction between Arabidopsis and *P. syringae*, *LOL2a* is upregulated at 12 and 24hpi, whereas *LOL1* is downregulated. The timing of these responses indicates an involvement of LOL2 and LOL1 in processes leading to a containment of HR cell death. Analysis of *lol1* and *lol2* mutants showed that LOL1 is negatively regulated by LOL2. Additionally, *lsd1* mutant plants harboring a *LOL1* antisense construct display reduced lesioning after treatment with BTH, *P. parasitica* or *B. cinerea*. Preliminary results suggest that the same phenotype can be achieved when *LOL2a* is overexpressed in *lsd1* mutants plants. Thus, the balance between LSD1, LOL1 and LOL2 appears to regulate cell death with LSD1 as a negative regulator and LOL1 as a positive regulator that can be negatively regulated by LOL2.

This work was supported by grants from the Swiss National Foundation (Schweizer Nationalfond) and the German Academic Exchange Service (Deutscher Akademischer Austauschdienst) to PE and NIH grant 1-R01-GM057171-01 to JLD.

174 A conditional mutant that affects guard cell division and flower development

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We are characterizing an *Arabidopsis* mutant whose guard mother cells fail to divide appropriately to yield the two guard cells of the stomata.The defect appears to be in cytokinesis, because the defective guard mother cells have partial or missing cell walls and are binucleate. The mutant is pleiotropic, and the expressivity of several facets of the mutant phenotype depends critically upon the growth conditions. The rosette size depends strongly upon the light cycle: under short days the mutant rosettes are indistinguishable in size from wild type rosettes, while under continuous illumination the mutant plants are severely dwarfed. Flower development depends strongly upon the growth temperature: at 22°C the mutants do not produce functional flowers, only very small buds or intermediate forms with sepals and carpels. When plants are grown at 16°C under continuous illumination flower development is normal: large amount of seed can be obtained from mutant homozygotes that produce no seed at 22°C. Plants grown at 22°C have few functional stomata, but plants grown at 16°C appear to have normal stomata. The mutant locus has been mapped to a 350 kb interval on chromosome I (at approx 66 cM, 17.6 Mbp). Given the pleiotropic phenotype, it is possible that more than one gene is affected in this EMS mutant. However, genomic southern blotting to date has not detected a deletion or chromosomal rearrangement within the map interval. Thus, this gene may represent a link between the pathways of cell division in guard mother cells and flower development.

175 Role of NPR1 Phosphorylation in Salicylic acid signaling

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Arabidopsis NPR1 is an essential regulator of Salicylic acid (SA) signaling. The *npr1* mutant is not only defective in SA-induced PR gene expression and establishment of systemic acquired resistance (SAR), but is also hypersensitive to excess amounts of SA. 0.5 mM SA in the growth media causes bleaching and growth arrest to *npr1* seedlings. Both *cpr5*/*npr1* and *cpr6*/*npr1* double mutants bleach in leaves and stems because they accumulate high levels of SA. Sequence comparison of NPR1 with its homologues reveals two highly conserved serine residues in the N-terminus that could be potential phosphorylation sites. Alanine or aspartic acid substitutions were made to these two serine residues and the mutated NPR1 gene ($NPR1^{S-A}$ or $NPR1^{S-D}$) was transformed into both wildtype and npr1 mutant plants, under the expression control of the CaMV 35S promoter. In *npr1* mutants, $NPR1^{S-A}$ restores SA-induced PR gene expression to a level similar to that of plants overexpressing wildtype NPR1 or NPR1S-D protein. However, $NPR1^{S-A}$ does not complement the SA hypersensitive phenotype in *npr1* mutant, while NPR1 and $NPR1^{S-D}$ both do. Moreover, overexpression of $NPR1^{S-A}$ in wildtype plants causes hypersensitivity to SA, indicating that $NPR1^{S-A}$ has a dominant-negative effect. These data suggest that NPR1 is phosphorylated at the N-terminal serine residues, and the phosphorylation is required for NPR1 to regulate tolerance to high concentrations of SA. The dominant-negative effect of $NPR1^{S-A}$ also suggests that protein-protein interaction might be involved in this NPR1 function.

176 The Arabidopsis mutant, *rhd2*, reveals a role for reactive oxygen species in root hair morphogenesis

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The Arabidopsis root has a simple and invariant structure and provides an excellent model system for the study of plant development. During differentiation of the root, specialised cells in the epidermis (trichoblasts) form root hairs - tip growing tubular shaped outgrowths. Three stages of root hair growth have been identified. The first is the formation of a bulge at the apical end of a trichoblast cell, tip growth then occurs from a distinct point on this initial bulge. The third stage of development is defined by an increased rate of tip growth. Root hairs on *root hair defective2 (rhd2)* mutants arrest after the first stage of growth. A new *rhd2* allele was identified in a Spm mutagenised population. Sequencing of the flanking regions revealed that *RHD2* encodes an *Arabidopsis thaliana* respiratory burst oxidase homolog (*At*rboh). The genes in this family are homologous to the β subunit ($gp91^{phox}$) of the mammalian NADPH oxidase flavocytochrome b_{558} which contains the entire electron transport chain from NADPH to oxygen. It has been postulated that Atrboh proteins produce reactive oxygen species (ROS), since NADPH oxidase produces ROS during electron transfer in mammals. The *rhd2* mutant lacks the high levels of ROS present in root hairs of wild-type plants supporting this view. Drug studies are being used to biochemically characterise the role of RHD2 in the production of ROS. The expression patterns and cellular localisation of RHD2 is presently being determined. Double mutant combinations with other mutants with defects in root hair elongation have been generated and are been used to characterise the role of RHD2 in root hair morphogenesis.

177 Reverse genetic and structural analysis of the SLATs

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We have identified 10 genes encoding a novel class of plant leucine-rich repeat proteins (LRRPs). The defining feature of these proteins is an internal LRR domain consisting of 8-11 copies of a signature leu-rich motif. Based on this motif structure and BLAST alignments, these proteins are more closely related to "Ras group" animal and fungal LRRPs than to any previously characterized plant LRR proteins. Ras group LRR proteins function in intracellular signal transduction, and some, such as C. elegans/human SUR8, interact directly with RAS. We have named this novel class of plant LRRPs "SLATs", for SUR8-like LRRs of Arabidopsis thaliana. Here, we present a comparative analysis of SLAT protein and gene structures and mRNA expression, and report on progress toward isolating T-DNA knock-out alleles of each of the *SLAT* genes. As a group SLATs share common structural features such as the LRR consensus motif and internal LRR domain. They are intracellular and have highly hydrophilic N- and C-terminal domains rich in charged amino acids and low-complexity stretches. These features distinguish SLATs from animal Ras group LRRPs and define them as a related, but plant specific, class of proteins. Despite their shared features, SLATs fall into 4 distinct families based on size, primary sequence, and gene exon/intron structure. Primary sequences vary considerably even within each family, with the most closely related SLATs sharing amino acid identity of only ~70%. Thus, the SLATs as a group are likely to have different functions. To investigate SLAT functions, we have initiated a screen for knockout mutants. To date we have identified T-DNA insertion alleles for 7 of the 10 *SLAT* genes and are in the process of obtaining homozygous mutant individuals and determining T-DNA insert numbers. Leu-rich repeats serve as protein:protein interaction domains, and the SLAT's structural features suggest that they function in intracellular signal transduction, interacting with other cellular components through their LRR domains. Phenotypic analysis of *slat* knockout mutants should help address this hypothesis and elucidate the biological functions of these proteins.- *Supported by NSF (IBN 9604344) and the Murdock Trust*

178 Distribution of fucose-containing cell wall polysaccharides in the roots of the mur1 mutant of Arabidopsis thaliana

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The *mur1* mutant of *Arabidopsis thaliana* is characterized by an absence of fucose in shoot-derived cell wall polysaccharides and a 40% reduction in fucose walls isolated from roots compared to wild-type plants. The mutant plants are defective in one of the enzymes required for the biosynthesis of GDP-L-fucose, the activated sugar precursor needed for biosynthesis of fucose-containing polysaccharides. The monoclonal antibody, CCRC-M1, which recognizes a fucose-containing epitope in cell wall polysaccharides, was used to determine the distribution of fucose-containing polysaccharides in roots of the *mur1* mutant. Immunolabeling with CCRC-M1 was carried out on *mur1* root tissue taken from seedlings 1 to 14 days post-imbibition. No labeling was observed in hypocotyls, shoots, or leaves. Immunofluorescent labeling of whole seedlings revealed that *mur1* root hairs were stained heavily by CCRC-M1, while the body of the root remained unstained or only lightly stained. Immunogold labeling revealed that CCRC-M1 labeling was restricted to specific regions of the root, or to specific cell walls and cell types. All cell walls at the apex of primary roots 2 days and older and in the apices of mature lateral roots were labeled with immunogold. The fucose-containing epitope is absent from 1 day old germlings and from lateral root buds. Labeling with CCRC-M1 decreases rapidly in cells that are undergoing rapid elongation growth such that, in the mature portions of both primary and lateral roots, only the pericycle and the outer walls of the epidermis are labeled. Wild-type lobeling, where all cell walls are strongly labeled by CCRC-M1, is restored within *mur1* roots by growing the mutant on media supplemented with fucose. The asymetric distribution of the fucosyl epitope suggests that more than one pathway for the biosynthesis of fucose exists in *A. thaliana*, and that these pathways exhibit different temporal and spatial patterns of expression. These results also imply a coordinated regulation between the Golgi-localized biosynthesis of cell wall polysaccharides and cytosolic components that supply the necessary precursors for that process.

179 HOBBIT, a component of the APC involved in control of cell division and cell fate ?

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The *HOBBIT* gene is required during early embryonic development for proper cell division first in a specific founder cell of the root meristem, the hypophysis, and later on in the basal part of the embryo. However, post-embryonicaly, *hobbit* mutant displays several complex phenotypes, such as defective meristems and absence of specific cell identities. This suggests that HOBBIT function is involved in controlling cell division patterns as well as acquisition of some cell fates.

Sequence analysis of the *HOBBIT* gene suggests homology with TPR-containing proteins involved in the Anaphase Promoting Complex (APC). This complex is responsible for targetting specific sets of substrates for proteolysis through an ubiquitination pathway, and controls metaphase/anaphase transition and metaphase exit. Expression analysis at transcript level reveals that *HOBBIT* is expressed ubiquitously during early stages of embryogenesis. However, in mature embryos and post-embryonically in root tips, transcripts are surprisingly detected only in some dividing cells, suggesting a cell cycle regulation of *HOBBIT* expression. In contrast, other related TPR subunits shows a ubiquitous expression pattern in all different cell cycle phases and at all developmental stages, similarly to their orthologs in other systems. To assess if HOBBIT function is mediated through its involvement in the APC, heterologous expression in yeasts deficient for this complex was performed, leading to partial rescue of their growing ability. Finally, HOBBIT potential relation with cell cycle control was analyzed using flux cytometry, revealing nuclear DNA contents that might reflect abnormal cell cycle progression.

Our results suggest that HOBBIT may be involved in targetting for proteolysis some specific substrates regulating either cell division progression or cell fate determination, as a subunit of the APC. Several strategies are currently in progress to identify such substrates.

180 *Arabidopsis SPIRAL1*gene controls cortical microtubule arrays.

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Mutations in *Arabidopsis SPIRAL1 (SPR1)* locus cause isotropic expansion of endodermal and cortical cells in root, etiolated hypocotyl, and dark-grown influorescent stem, and induce right-handed spiral in epidermal cell files of these organs. Interestingly, addition of either microtubule-depolymerizing drug propyzamide at 1 µM or microtubule-stabilizing drug taxol at 0.3 µM in the culture medium was found to completely suppress the cell expansion defects of *spr1*. Wild-type root epidermal cells had cortical microtubule arrays that were aligned almost transverse to the long axis of the cell, while *spr1* epidermis had left-handed helical arrays. *SPR1* was cloned by a map-based approach, and found to encode a plant-specific novel protein of low molecular weight. Transgenic *Arabidopsis* plants overexpressing SPR1 had somewhat larger leaves and thicker stems than wild type, bolted late, and showed increased tolerance to propyzamide. SPR1-GFP fusion protein expressed under the control of the *SPR1* promoter complemented *spr1* mutant phenotypes. In hypocotyl epidermal cells of the transgenic plants, GFP fluorescence was localized as fibers at cell's cortical region, which presumably represent cortical microtubules. We propose that SPR1 stabilizes cortical microtubules and influences their dynamic properties.

181 Isolation and characterization of an *Arabidopsis vw331* mutant defective in root and hypocotyl elongation

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In *Arabidopsis*, post-embryonic growth of hypocotyls takes place in the absence of cell division. Difference in length between hypocotyls of dark-grown seedlings (about 25 mm) and those of light-grown ones (about 1 - 3 mm) is due to difference in degree of cell elongation in hypocotyls. Although cell elongation in hypocotyls is known to be regulated by the light conditions and plant hormones, the mechanisms of its regulation and of cell elongation itself are not well understood.

Here we report a *vw331* mutant whose hypocotyl elongation is defective in the dark condition. Light-regulated hypocotyl elongation in *vw331* is as normal as that in wild type. These indicate *vw331* has a defect in hypocotyl elongation associated with the dark-grown developmental program. On the other hand, in roots of *vw331*, cell elongation is inhibited irrespective of light or dark condition. In *vw331*, the conditional defect in hypocotyls and the constitutive defect in roots are associated with abnormal bulging of the surface of the epidermal cells due to aberrant swelling of epidermal, cortex, and endodermal cells, suggesting that *vw331* is defective in regulated expansion of the cell wall. Moreover, cell wall gaps were often observed in transverse sections of roots and dark-grown hypocotyls of *vw331*, which may also be representing the cell wall defect in *vw331*. These observation indicate that *VW331* gene is necessary for controlled expansion of the cell wall required both for the light-dependent hypocotyl cell elongation and for the light-independent root cell elongation.*VW331* gene was mapped on the top of the chromosome I. Further mapping is in progress.

182 Glucose trimming of the core N-glycan is required for cellulose biosynthesis

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Cellulose has long been proposed to play a crucial role in the control of cell elongation and plant morphogenesis. This has been supported by recent genetic analysis of the cellulose deficient *rsw1-1* seedling mutant. To look for more severe mutations affecting cell elongation, we performed a genetic screen for mutants with altered embryo shape. Two genes that are required for correct polarity of cell expansion are presented here. Both mutants are radially swollen during embryogenesis, and have reduced levels of cellulose.

The first of these mutants is a null allele of the RSW1 catalytic subunit of cellulose synthase, *rsw1-2*. The second group of mutants are allelic to *knopf*, which was previously described as a seedling mutant with altered shape. We have cloned the *KNOPF* gene based on its map position. KNOPF has 30% identity with α-glucosidase I, the first glucose trimming enzyme in the processing of N-linked glycans. *knopf* embryos lack α-glucosidase I activity, and are blocked in N-glycan processing.

Trimming of N-glycans has previously been shown to be required for chaperone-mediated protein folding in the endoplasmic reticulum. Since the cellulose synthase RSW1 contains several N-linked glycosylation motifs, this suggested that misfolding and subsequent degradation of RSW1 might explain the decrease in cellulose in *knopf* embryos. We demonstrate that RSW1 is in fact not N-glycosylated, and that Wt and *knopf* embryos contain equal amounts of RSW1. Thus, the decrease in cellulose in *knopf* embryos is not due to degradation of RSW1.

We present a simple biophysical model to explain the altered shape of *knopf* and *rsw1-2* mutants during embryogenesis. Our results demonstrate that trimming of the core N-glycan is required for cellulose biosynthesis, and indicate that mutations affecting cellulose biosynthesis can be recovered during embryogenesis.

183 Characterization of lignification mutants in *Arabidopsis Thaliana*

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Arabidopsis has now become a model plant for lignification studies as mature stem displays a high lignification rate and typical lignins from Angiosperms (G and S units). The T-DNA insertion library of Versailles was screened to isolate tagged mutants affected in monolignols biosynthesis pathway. The goal of this work is to compare the phenotypes of the Arabidopsis mutants to those observed in other species obtained by antisense or chemical mutagenesis strategies in order to valid this model system.

One line displays a translationnal fusion between the caffeate O-methyltransferase (COMT) gene and the coding sequence for β-glucuronidase. The COMT is preferentially involved in the synthesis of syringyl units (S units) of lignins. The GUS fusion allowed us to monitor the expression pattern of this gene throughout the entire development of the plant, in addition to analyses of lignin quality. The mutant lignins completely lack S unit but contain its direct precursor in small amount, the 5-OH-G unit.

Lines mutated in genes coding for cinnamyl alcohol dehydrogenases were identified. CAD catalyses the reduction of cinnamaldehydes to monolignols. One mutant shows important perturbations in lignin composition whereas no lignin modifications are observed in another.

These phenotypes in *Arabidopsis* mutants are very similar to those obtained in other species (tobacco and poplar antisense, maize mutant bm3, …). These results are in favor of pursuing this work on *Arabidopsis* for lignification study. The screening of the insertion library is now carried out in order to identify new mutants of this biosynthetic pathway (genes involved in polymerisation, regulation,…). The obtention of several lignification mutants in a sole species will allow us to better understand this pathway and, especially its regulation.

184 Cell polarity signaling in *Arabidopsis* involves a Brefeldin A-sensitive auxin influx pathway

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Cell polarity in the *Arabidopsis* root epidermis is reflected by polar hair outgrowth from hair cells (trichoblasts) (1). Hairs initiate basally, oriented towards the root tip where auxin accumulates at maximum concentration (2). Here we report, that auxin influx carrier *AUX1* (3-5) function contributes to polar basal hair initiation, since *aux1* mutants display apically shifted hairs and double hair formation from trichoblasts. The phenotypes are rescued when the wild-type genomic *AUX1* sequence with an insertion for a hemagglutinin (HA) epitope-tag is introduced into the *aux1* mutant background. Exogenous application of the synthetic auxin influx carrier substrate 2,4-dichlorophenoxyacetic acid (2,4-D) (6) enhances basal hair initiation and *aux1* mutants are resistant to this 2,4-D effect. Thus, *AUX1* function is required for 2,4-D-induced trichoblast basalization. The vesicle trafficking inhibitor brefeldin A (BFA) (7,8) causes specific trichoblast polarity defects at low concentrations. The BFA-induced phenotypes are similar to but stronger than those observed for *aux1* mutants. *aux1* mutations confer resistance against BFA action on trichoblast polarity demonstrating that *AUX1* activity is required for BFA-induced polarity changes. Immunolocalization of the functional HA-AUX1 protein reveals that BFA inhibits plasma membrane localization of AUX1, further indicating membrane trafficking of the influx carrier as a target for BFA-interference. Accordingly, BFA inhibits action of exogenously applied 2,4-D on trichoblast polarity. Finally, BFA alters expression of different auxin-inducible reporters in the root tip. Our results reveal AUX1 as one component of a novel BFA-sensitive pathway polarizing epidermal cells towards an auxin maximum.

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185 A symbiosome nodulin of soybean, Nodulin-24, is targeted to the vacuole

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Nodulin-24 is a membrane protein of soybean root nodules. It was shown to have a cleavable signal sequence and localized to the membrane enclosing the symbiotic bacteria. For understanding the targeting process of nodulin-24, nodulin-24 cDNA was fused to ¥â-glucuronidase (GUS) gene and the fused construct was introduced into yeasts. Subcellular fractionation and marker enzyme assays were performed for localization of the fusion protein; The GUS activity was concentratedly found in the P13 fraction which supposedly contains ER and vacuoles. Isolated vacuoles by discontinuous ficoll gradient centrifugation also have high GUS activity. It was concluded that the fusion was targeted to vacuole in yeast. Vacuolar targeting of nodulin-24 in yeast may suggest that the symbiosome is an organelle equivalent to vacuoles. For nodulin-24 targeting in Arabidopsis, the fusions were introduced into Arabidopsis and the analysis of the transgenic plants are underway. The transient expression of nodulin-24/green fluorescent protein (GFP) fusion is also being performed.

186 molecular genetic characterization of a calcium binding protein in photomorphogenesis of Arabidopsis seedlings

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As the major photosensory receptors in plants, cryptochromes and phytochromes often regulate the same photomorphogenetic responses. The molecular mechanism underlying functional interactions of cryptochromes and phytochromes remains unknown. In an attempt to identify the downstream components in cryptochromes signal transduction pathway, we have identified an Arabidopsis photomorphogenetic mutant, sub1, which exhibits hypersensitive responses to blue light and far-red light. Genetic analyses indicate that SUB1 functions as a component of a cryptochrome signaling pathway and a modulator of phyA signal transduction. The SUB1 gene is cloned and encodes an EF-hand calcium-binding protein that suppresses light-dependent accumulation of HY5, a transcriptional factor that positively regulates light responses (Guo et al. Science (2001) 291:487-490). Further studies revealed that sub1 is primarily expressed in hypocotyls and stem tissues, but not found in seeds, roots or leaves. SUB1 protein is enriched in the nuclear periphery as well as cytoplasm, suggesting sub1 might regulate the trafficking of several nuclear proteins, including COP1 and PHYA. A putative model on cryptochrome signaling pathway is discussed based on the above observations.

187 Tissue-Specific and Developmental Regulation of MT Isoforms in *Arabidopsis*

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Metallothioneins (MTs) are cysteine-rich proteins that bind metals via thiol clusters. While it is established that MTs are required for heavy metal tolerance in fungi and animals, the role of MTs in plants is not yet clear. In *Arabidopsis*, 7 actively expressed MT genes have been identified and these can be grouped into 4 classes. To further investigate tissue-specific and developmental regulation of MT genes, we produced transgenic *Arabidopsis* plants containing GUS reporter genes under the control of different MT promoters. The *MT1a* and *MT2a* promoters are highly expressed in roots and leaves, respectively. In contrast, the *MT2b*-GUS reporter is expressed in all organs. In roots, *MT1a* is primarily expressed in the cortex, whereas *MT2b* is expressed in phloem. *MT2a* expression in roots is restricted to the root tip. In leaves, *MT1a* and *MT2b* are expressed predominantly in phloem. In response to copper treatment, expression of MT genes in phloem is enhanced both in roots and leaves, and especially in trichomes of expanding leaves. Transcription of MT genes is also regulated during development. Elevated expression of MT-GUS reporter genes in leaves and roots increased as these organs aged and senesced. Expression of MTs was observed in trichomes on older plants. Based on these observations, we propose that MTs may function as a carrier for metal transport in the phloem. In this capacity, MTs can reduce toxicity of metal, such as copper. In addition, trichomes may be a site for deposition and storage of excess metals.

188 Two SECY homologs involved in thylakoid protein transport have essential, non-redundant roles in embryo development

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Imported proteins are translocated across the plastid thylakoid membrane by four different pathways. The Sec-dependent pathway involves a trimeric SecYEG complex that functions as a protein-conducting channel. To study the contribution of the Sec-dependent pathway to plastid biogenesis, we have screened for insertional mutations in the two *SECY* homologs in the Arabidopsis genome. Two mutant alleles of *SECY1* and one mutant allele of *SECY2* have been identified. Individuals carrying the *secY2* allele in the homozygous condition arrest at the globular stage of embryo development. Individuals carrying the *secY1-1* and *secY1-2* alleles in the homozygous condition also arrest during embryo development. These results indicate that both SECY1 and SECY2 play essential, non-redundant roles in early embryo development. To gain further insight into the functions of SECY proteins in Arabidopsis, we are also comparing the expression patterns of *SECY1* and *SECY2* in various tissues and at different developmental time points.

189 Arabidopsis ABA responses are regulated by a HD-Zip protein, a target of the protein phosphatase 2C ABI1

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The Arabidopsis protein phosphatase 2C (PP2C) ABI1 is a key component of the ABA signal transduction pathway. By analysing protein/protein interactions we have identified a transcription factor of the homeodomain-leucine zipper (HD-Zip) class as a new component of the ABA signalling pathway. The transcription factor interacts with ABI1 in yeast and in vitro. The enzymatically less active mutant protein abi1 and an inactive version of ABI1 were impaired in binding, indicating that the interaction is dependent on the protein phosphatase domain of ABI1. The transcription factor recognises a pseudopalindromic DNA sequence in vitro, which is also present in its own promotor. Moreover this cis-element mediates gene activation in transient expression analysis in Arabidopsis. In addition, transgenic Arabidopsis plants ectopically expressing the transcription factor revealed altered ABA responses. From these data we conclude that the transcriptional factor represents a component of the ABA signalling cascade acting downstream of ABI1.

190 Chacterization of the *auxin-resistant6* mutants

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The *auxin-resistant6* mutants display a variety of alterations in development and physiology that are consistent with an altered auxin response throughout the plant (Hobbie et al., 2000, *Development* **127**, 23-32). Heteozygous mutants show altered growth habit, reduced root gravitropism, and auxin-resistant root growth and hypocotyl elongation. Homozygous mutants generally show aberrant embryonic development, leading to seedlings that consist of cotyledons joined to a basal stub but lacking roots and hypocotyls. Our recent results include an analysis of the phenotype of double mutants between *axr6-2* and the dominant mutation *axr2*, which is consistent with the two mutations showing largely additive effects. Analysis of the hypocotyl cells of light-grown mutant and wild-type seedlings shows that the *axr6* heterozygous mutants have an increased number of smaller cells compared to wild type, resulting overall in hypocotyls of about the same length as wild-type. Measurements of auxin transport in the stems of *axr6* heterozygous plants indicate little difference from wild type.

Lines of viable plants that are homozygous for the *axr6-2* mutation have been identified. These homozygous viable plants produce progeny that consist of 80-95% rootless seedlings and 5-20% seedlings that develop roots. The viable homozygotes appear to be more strongly affected in all aspects of the mutant phenotype than the heterozygous mutants. The existence of these plants suggests that the *axr6-2* mutation results in the level of a crucial developmental signal or pathway that is very close to a threshold for proper development of the basal structures (root and hypocotyl). Chance variations may sometimes put an individual embryo into the normal range.

The map position of the *AXR6* gene has been defined to a region of approximately 60,000 bp on the short arm of chromosome 4. This region contains no Aux/IAA or ARF gene family members. Testing of candidate genes is in progress.

These results expand our knowledge of the development and physiology of the mutants and represent significant progress towards molecular characterization of the *AXR6* gene.

191 The Sugar-Insensitive Mutant *sis2* Germinates on Paclobutrazol, a Gibberellin Biosynthesis Inhibitor

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Sugar levels have been postulated to affect plant developmental processes, including germination, time to flowering, and carbon partitioning. However, little is known about the mechanisms by which plants sense and respond to sugar. Gaining an understanding about these mechanisms may eventually enable the improvement of crop yield.

The *sugar-insensitive2 (sis2)* mutant was isolated from a pool of EMS-mutagenized seeds based on its ability to form true leaves and a substantial shoot system on 0.3M glucose. Wild-type seeds germinate on 0.3M glucose, but further development is arrested. The *sis2* mutation does not affect sugar transport, as not all sugar responses in the *sis2* mutant are altered.

As work from several labs indicates that sugar-response mutants may show alterations in phytohormone response or metabolism, I characterized the response of *sis2* to several phytohormones (and to some of their respective inhibitors). The *sis2* mutant demonstrates wild-type root length inhibition on auxin, cytokinin, and methyl-jasmonate. Interestingly, *sis2* is insensitive to the inhibitory effects of paclobutrazol, a gibberellin biosynthesis inhibitor, on seed germination. In contrast, *sis2* demonstrates almost wild-type sensitivity to paclobutrazol at later developmental stages.

As previous work shows that ABA-deficient, ABA-insensitive, ethylene-constitutive response, and ethylene-overproduction mutants also germinate on paclobutrazol, I tested the response of *sis2* to these phytohormones. The *sis2* mutant demonstrates wild-type germination on ABA, and wild-type hypocotyl elongation on the ethylene and brassinosteroid biosynthesis inhibitors AVG and brassinazole, respectively. The *sis2* mutant has a very subtle wilty phenotype (an ABA response). Experiments to directly measure ABA levels in the *sis2* mutant are currently being performed.

As the pathway through which the *sis2* mutation confers sugar and paclobutrazol resistance remains unclear, cloning the *SIS2* gene should help define its function. Previous work has mapped the *SIS2* gene to the bottom of chromosome 1, 11.4 cM away from the nga111 marker.

Supported by Department of Energy Biosciences Program Grant #DEFG03-00ER15061.

192 The effect of mutations in the exportin-t homologue PAUSED on shoot development

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In Arabidopsis, the transition from the juvenile to the adult vegetative phase is marked by changes in leaf shape and the appearance of trichomes on the abaxial surface of the leaves. *paused* (*psd*) was isolated as a mutation which causes the appearance of adult characteristics in the first two leaves. Examination of *psd* mutant plants revealed that the timing of the juvenile to adult transition is not affected, but there is a delay in leaf initiation which causes the first leaves to be produced during the adult phase. This delay in leaf production is associated with the death of a small group of cells in the central zone of the shoot apical meristem. We have cloned the *PSD* gene and shown that it is a member of the importinβ family of nuclear import/export factors. PSD is most similar to the *S. cerevisiae* protein Los1p, which is involved in the export of tRNA. We will report on the molecular characterization of *PSD* and its potential involvement in tRNA export.

193 D-type cyclins in plant cell cycle control

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In contrast to animals, plants have indeterminate growth and thus require the ability to alter their growth rate or pattern in response to environmental or developmental signals. Cell division in plants is concentrated in specialised regions called meristems. A small group of cells in the meristem divides to continually supply cells which are directed to organ formation. The relationship between cell division and the growth pattern of the whole plant is still not fully understood, in order to help in this we need to determine how the cell cycle in plants is controlled. The major players in the plant cell cycle have been identified and are basically similar to the components of the mammalian cell cycle, however, their specific roles and the manner in which they are regulated appear to be plant-specific. D-type cyclins operate at the G1 to S phase transition of the cell cycle and there is evidence that they play an important role in controlling commitment to cell division as well as mediating responses of plant cells to extracellular signals such as sucrose and cytokinin. Manipulation of the levels of D-type cyclins will help us to understand their role in the cell cycle and how this is linked to the overall growth of plants. We have overexpressed *Arabidopsis* cyclin genes in both Landsberg erecta and Columbia ecotypes of *Arabidopsis* . The phenotypes of the transgenic plants are reported together with preliminary analysis of the phenotypes.

194 SPATIAL EXPRESSION OF MYROSINASE GENE PROMOTER TGG1 IN SEEDLING AND VEGETATIVE ARABIDOPSIS

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Myrosinase (EC 3.2.3.1) also known as thioglucoside glucohydrolase (TGG), catalyses the hydrolysis of glucosinolates into glucose, sulfate and a wide range of products. The products are toxins to a wide range of microbes and generalist herbivores but stimulate oviposition and feeding of specialist insects. In *Brassica napus* there are multiple forms of myrosinase genes in contrast to *Arabidopsis thaliana* where there are two known functional myrosinase genes, TGG1 and TGG2. 23 different types of glucosinolates have been isolated from *A. thaliana*.

We have made transgenic *A. thaliana* plants containing the TGG1 and TGG2 promoters fused to GUS or GFP reporter genes to study the spatial distribution and level of myrosinase expression in response to different stimuli such as environmental conditions and insect challenge. Within the TGG1 GFP fusion genes myrosinase N- and/or C-terminal encoding sequences were included to investigate their role in compartmentalization myrosinase at subcellular level.

The TGG1 promoter shows specificity towards guard cells and phloem cells in *A. thaliana* seedlings and vegetative plant. In the flower stalk all guard cells show TGG1 promotor expression while positive phloem cells appear in a discontinuous cell pattern. Transverse sections show that phloem cells with GUS and GFP expression are phloem parenchyma cells. Together with guard cells this special population of phloem cells show reactivity towards our polyclonal myrosinase antibody K089, suggesting that in *A. thaliana* both are myrosin cells. Cells with a phenotype similar to S-cells, recently reported to be rich in glucosinolates, are located next to the myrosinase containing phloem cells. Substrate and catalytic enzyme therefore appear to be contained in separate but associated cells in the *A. thaliana* flower stalk.

Myrosinase TGG1 promoter activity is higher in young developing tissue than older tissue. In young tissues a high myrosinase level may be necessary for proper protection towards microbes and insects due to lower physical strength barriers.

195 Topical application of harpin induces plant defense responses in *Arabidopsis thaliana* through both the JA/ethylene-dependent and SA-dependent pathways

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Harpin, encoded by the HrpN gene of *Erwinia amylovora*, has previously been demonstrated to be an elicitor of the hypersensitive response, but its precise mode of action is not yet known. Topical application of harpin protein confers resistance to a wide range of plant pathogens. To start to understand the mechanisms of harpin-induced defense responses, we assessed harpin effects on expression of marker genes for the salicylic acid (SA)- and jasmonic acid (JA)/ethylene-dependent defense pathways. Arabidopsis lines with mutations in these defense pathways were used to verify that harpin-induced changes in marker gene expression were mediated by these pathways. Activation of the SA-dependent pathway is indicated by induction of PR1, PR2, and PR5 expression and the JA/ethylene-dependent pathway by induction of PDF1.2 expression. Topical application of harpin resulted in the dose dependent induction of these marker genes indicating roles for both pathways in harpin-mediated defense responses. This was in contrast to the effect of the salicylic acid analogue benzothiadiazole (BTH) which induced PR1 expression but had no effect on PDF1.2 expression. The ability of harpin to induce defense gene expression was determined in *npr1* and *eds5* plants in which the SA signaling pathway is disrupted and in *ein2* and *jar1* plants containing mutations affecting the JA/ethylene pathway. PR1 induction by harpin was blocked in *npr1* and *eds5* plants, indicating that harpin signaling acts through a functional SA-dependent pathway. Likewise, harpin treatment of *ein2* did not result in PDF1.2 induction, confirming that harpin signaling also occurs through the JA/ethylene dependent pathway. The finding that harpin activates at least two defense pathways to induce defense gene expression provides initial insight into the capacity of harpin to induce resistance to a broad range of pathogens and indicates an upstream point of interaction by harpin with defense responses.

196 Genetic clues to unravel the cpSRP pathway for the targeting of LHCPs to thylakoids

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The chloroplast signal recognition particle (cpSRP) is one of the four pathways which target proteins to thylakoids. It shares similarities with cytosolic SRP complex that co-translationally targets proteins to endoplasmic reticulum or periplasmic membrane. Both contain a 54kD protein with GTPase activity and are dependent on a second GTPase protein identified as the -subunit of the SRP receptor and its chloroplast homologue, cpFtsY. CpSRP differs from cytosolic SRP by the absence of RNA component and the presence of a 43kD protein. Furthermore, the 43 and 54kD proteins cpSRP complex functions post-translationally. The membrane component of the cpSRP includes ALB3, an homologue of the yeast OXA1 component required for the assembly of the mitochondrial cytochrome c oxidase. Biochemical and genetic studies have established that the most abundant nuclear-encoded thylakoid protein family, the light-harvesting chlorophyll proteins (LHCPs), uses this pathway. Previous analyses of chaos and ffc cpSRP mutants, respectively deficient in cpSRP43 and cpSRP54, revealed that half of the LHCPs is integrated into the thylakoids. This suggested the presence of an alternative pathway for LHCP targeting. This hypothesis was ruled out by the analysis of the double mutant which indicates an additive effect of the chaos and ffc mutations. The near-total loss of LHCPs in the double mutant demonstrated that cpSRP is the predominant targeting pathway for these proteins. In addition, analysis of the plantlets reveals that cpSRP is required for the import of nuclear proteins distinct from LHCP. We have identified a ftsY mutant, and the plantlets differ from chaos and ffc, suggesting new insights into the cpSRP mechanism.

197 Genetic studies of chloroplast protein import in *Arabidopsis*

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Most chloroplast proteins are encoded in the nuclear genome, translated on cytosolic ribosomes, and imported post-translationally into chloroplasts. Chloroplast protein import is mediated by the coordinate action of translocon protein complexes in the outer and inner envelope membranes of chloroplasts. Biochemical studies of isolated pea chloroplasts have greatly enhanced our understanding of the chloroplast protein import mechanism, and resulted in the identification of many components of the import apparatus – components of the outer and inner envelope membrane complexes are referred to as Toc (translocon at the outer envelope membrane of chloroplasts) and Tic proteins, respectively. A number of limitations of the biochemical approach, however, have led to the recent emergence of *Arabidopsis* as an excellent alternative model system for studying chloroplast protein import. One major advantage of *Arabidopsis* is that it is amenable to the application of genetics. By identifying mutant plants with defects in Toc/Tic genes, one can study the roles of translocon components *in vivo*. My laboratory has used a variety of forward and reverse genetic strategies to identify *Arabidopsis* mutants with chloroplast protein import defects. *Plastid protein import 1* (*ppi1*) is null for Toc33, has significant chloroplast protein import defects, and was the first such mutant to be identified. We are currently engaged in experiments to characterize this mutant and others, and expect that our results will improve understanding of the import mechanism, how it is regulated, and the role played by non-proteinaceous envelope components.

198 Genetic dissection of RPP5-activated defence responses in Arabidopsis

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We are investigating how the race-specific resistance gene *RPP5* translates perception of downy mildew (*Peronospora parasitica* isolate Noco2) attack into effective defense. To identify the components that cooperate with *RPP5* to achieve disease resistance, we are isolating mutants that are defective in *RPP5*-function. We are especially interested in mutations that partially suppress *RPP5*-mediated defence, as these may prove critical in dissecting the intricate signalling network(s) controlling resistance.

Approximately 316,000 M2 EMS-mutagenized Landsberg-*erecta* seedlings have been screened, yielding 258 candidate partially susceptible (PS) mutants. The first 155 of these have been carefully progeny tested, yielding an intial 13 PS mutants on which this project focused. Complementation testing revealed six complementation groups, including two that may represent previously undescribed genes. For one of these, PS 138, we have localized the mutation to a 55 kbp region on the lower arm of Chromosome 2 and mutation detection analysis and complementation testing are underway. The remaining four PS complementation groups include the previously described *pad 4* (4 alleles), *rpr2*/*rar1* (1 allele, see poster at this meeting by Muskett P. et al), *sid2*/*eds16* (1 allele) and most likely *rpp5* (4 semi-dominant, partial-loss-of-function mutants). During this screen, we have also identified up to 81 total-loss-of-function mutations, which are completely susceptible to Noco2 attack. Analysis of the first 37 mutants in this class revealed that 28 are *rpp5* alleles, 2 *eds* alleles (*eds 1-9,10*), 3 *rpr1* alleles (*rpr1-2,3,4*, see poster at this meeting by Austin, M. J. et al), and 4 *rpr2*/ *rar1* alleles (*rpr2-2,3,4,5*). Progeny testing of the remaining 103 candidate partially susceptible mutants and 44 totally susceptible mutants is underway.

199 ADL1Ap and ADL1Ep, Two Isotypes of 68 kDa Arabidopsis Dynamin Like Protein are Involved in Plant Cell Morphogenesis

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The 68 kDa dynamin related protein ADL1Ap is required for multiple stages of plant development. In addition to *ADL1A*, the Arabidopsis genome contains four additional open reading frames that encode members of the *ADL1* family. However, RNA blot analysis showed that only *ADL1A*, *ADL1C* and *ADL1E* are transcribed. To address the functions of *ADL1A&ADL1E*, null mutants of *ADL1A* and *ADL1E* were isolated. Homozygous mutation of *ADL1A* conferred phenotypes of abnormal embryo development and conditional seedling lethality. In the presence of sucrose, homozygous *adl1A* seedling survived and appeared to developed normal (Kang *et al*., 2001). However, the leaves of mature *adl1A* homozygous plants have fewer trichomes than wild type and the trichomes have smaller number branches than corresponding trichomes in wild type. Papillae on stigmatic tissues of mutants never elongate. To analyze the expression of *ADL1A*, *ADL1A* promoter::β-glucuronidase reporter gene (GUS) activity was assayed. *ADL1A* promoter was active in embryo, cotyledons, at the site of trichome initiation, expanding papillae, and top of the stigma. The tissue specificity of *ADL1A* promoter::GUS expression is consistent with the phenotypes of *adl1A* homozygous mutants. Disruption of *ADL1E* did not cause any visible defect but the mutation of both *ADL1A* and *ADL1E* resulted in embryo lethality. *ADL1E* promoter::GUS expression indicated that *ADL1E* expression profile was almost identical to that of *ADL1A*. Our data suggest that *ADL1A* is involved in plant cell morphogenesis including papillae elongation and trichome development and that *ADL1A* and *ADL1E* have partially overlaping function.

200 RIN2 interacted with an Arabidopsis disease resistance gene, RPM1 encodes RING finger-type ubiquitin ligase.

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To dissect the RPM1-mediated disease resistance signal transduction pathway, we identified RPM1 interacters (RIN) by yeast two hybrid screening using the RPM1 fragments as baits. An RPM1 interacter, RIN2, contains putative transmembrane domains, a putative cytoplasmic RING finger domain and a novel C-terminal domain (which we term the DL domain) that is sufficient for interaction with the N-terminal domain of RPM1, including the coiled-coil domain. Recently, the RING finger proteins have been reported to function as E3 ubiquitin ligase, interacting with substrates to be ubiquitinated and targeted to the proteasome. Thus, RIN2 is suggested to encode an ubiquitin E3 ligase. Previously, we localized RPM1 as a peripheral plasma membrane, and noted that it is degraded just before the onset of the HR. This might be necessary to limit the spread of HR cell death beyond the cells in close proximity to the invading pathogen. The degradation of RPM1 is proteasome-dependent, suggesting that the RPM1 degradation may be involved in the ubiquitin ligase complex. To analyze whether RIN2 functions as E3 ligase of RPM1 through a direct interaction with the DL domain, we generated glutathione-S-transferase (GST) fusion proteins with RING domain and / or DL domain of RIN2. The GST-RIN2 protein itself is ubiquitinated in an E2 dependent manner. The RING domain of RIN2 is sufficient for an ubiquitin ligase activity. A possibility whether RIN2 can function as E3 ligase of RPM1 will be discussed.

This work is supported by NSF grant IBN-9724075 to JLD.

201 Trafficking of Phosphatidylinositol 3-Phosphate from the trans-Golgi Network to the Lumen of the Central Vacuole in Plant Cells

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Very limited information is available on the role of phosphatidylinositol 3-phosphate (PI[3]P) in vesicle trafficking in plant cells. To investigate the role of PI(3)P during the vesicle trafficking in plant cells, we exploited the PI(3)P-specific binding property of the endosome binding domain (EBD)(amino acids 1257 to 1411) of human early endosome antigen 1, which is involved in endosome fusion. When expressed transiently in Arabidopsis protoplasts, a green fluorescent protein (GFP):EBD fusion protein exhibited PI(3)P-dependent localization to various compartment-such as the trans-Golgi network, the prevacuolar compartment, the tonoplasts, and the vesicles in the vacuolar lumen- that varied with time. The internalized GFP:EBD eventually disappeared from the lumen. Deletion experiments revealed that the PI(3)P-dependent localization required the Rab5 binding motif in addition to the zinc finger motif. Overexpression of GFP:EBD inhibited vacuolar trafficking of sporamin but not trafficking of H+-ATPase to the plasma membrane. On the basis of these results, we propose that the trafficking of GFP:EBD reflects that of PI(3)P and that PI(3)P synthesized at the trans-Golgi network is transported to the vacuole through the prevacuolar compartment for degradation in plant cells.

202 AtVPS34, the PI3-Kinase that is responsible for production of PI(3)P at the TGN

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Phosphatidylinositol 3-phosphate [PI(3)P] has been known to traffic from the *trans*-Golgi network (TGN) to the lumen of the central vacuole in Arabidopsis. However, Phosphatidylinositol 3-kinase (PI3-kinase) that is involved in production of PI(3)P at the TGN has not been identified yet in Arabidopsis. Here, we present evidence that AtVPS34, a homolog of yeast Vps34p is the PI3-kinase. We isolated an Arabidopsis mutant with a T-DNA insertion at the At*vps*34 gene. The homozygote of the mutant was lethal. When we examined trafficking of PI(3)P in the heterozygote using GFP:EBD, a specific marker for PI(3)P, only half amount of GFP:EBD was transported to the central vacuole, compared with the wild type. This result suggests that the amount of PI(3)P produced in the heterozygote may be half of that in the wild type. In addition, we also find that when examined by the yeast two hybrid system, AtVPS34 interacts with AtVPS15, a homolog of yeast Vps15p, as in the case of Vps34p and Vps15p in yeast, suggesting that the mechanism for the targeting and activation of AtVPS34 to the TGN may be similar to that in yeast.

203 Increased Size Exclusion Limit of Plasmodesmata in Mutant Embryos of Arabidopsis thaliana

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Plasmodesmata provide routes for communication between plant cells by interconnecting the cytoplasm of adjacent cells. Their function and structure have been extensively studied, yet, a definitive functional protein component specific to plasmodesmata remains to be identified. In a genetic screen, we isolated *Arabidopsis* embryos carrying mutations of altered plasmodesmata function, designated *increased size exclusion limit of plasmodesmata (ise)*. These novel mutants showed a larger size exclusion limit of plasmodesmata when examined by a probe-movement assay. *ise* mutant embryos allowed the movement of fluorescently labeled 10 kDa dextrans between cells at the mid torpedo stage, whereas wild-type embryos did not allow the trafficking of the 10 kDa dextrans through the plasmodesmata. The morphology of the *ise*mutants discussed here, *ise1* and *ise2*, resembled that of the wild-type during embryo development. *ise2*was allelic to the previously characterized mutant *emb25* which gene maps to position 100 cM on chromosome I. We mapped the *ISE1* gene to region between two overlapping BAC clones, T12C24 and F13K23, spanning a 106.7 kb interval, located near position 10.5 cM on chromosome I using simple nucleotide polymorphisms (SNPs) as PCR-based biallelic markers.

204 The protein encoded by oncogene 6b from Agrobacterium tumefaciens interacts with a tobacco nuclear protein

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The *6b* gene in the T-DNA from *Agrobacterium tumefaciens* has oncogenic activity in plant cells, inducing tumor formation, the phytohormone-independent division of cells and altered leaf morphology in tobacco and Arabidopsis. The product of the *6b* gene appears to promote some aspects of the proliferation of plant cells but the molecular mechanism of its action remains unknown. We report here that the 6b protein associates with nuclear-localized protein NtSIP1 (Nicotiana tabacum 6b-interacting protein 1) of tobacco. NtSIP1 appears to be a transcription factor since its predicted amino acid sequence includes two regions that resemble, respectively, a nuclear-localization signal and a putative DNA-binding motif, which is similar in terms of amino acid sequence to the tri-helix motif of rice transcription factor GT-2. A fusion protein composed of the DNA-binding domain of yeast GAL4 protein and 6b protein activated the transcription of a reporter gene under the control of the GAL4 UAS-fused promoter in tobacco cells. Furthermore, nuclear localization of green fluorescent protein-fused 6b protein was enhanced by NtSIP1. A cluster of acidic residues in 6b protein appeared to be essential, for nuclear localization and for transactivation, as well as for the hormone-independent growth of tobacco cells. We propose that 6b protein might act in the proliferation of the plant cells through an association with NtSIP1.

205 A group of functionally redundant MAPKK Kinases that regulate cell division

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We have used a reverse-genetic approach to investigate the function of a group of three MAPKK Kinase genes: ANP-1, ANP-2, and ANP-3. T-DNA insertional mutants were isolated for each gene, and we observed that all of the single-mutant plants were phenotypically normal. Two of the three double-mutant combinations displayed mutant phenotypes, however; and the triple-mutant combination was found to be lethal in both gametes. Analysis of the double-mutant plants using transmission electron microscopy revealed the presence of cell wall stubs and bi-nucleate cells, indicating that cell division is disrupted in the mutant plants. We also utilized genome-wide expression analysis to compare anp2/anp3 double-mutant plants with wild-type. This experiment demonstrated that numerous genes involved in pathogen and stress responses are up-regulated in the double-mutant plants. Taken together, our results indicate that the ANP genes constitute a group of collectively essential kinases that positively regulate cell division and may negatively regulate stress responses.

206 dab5, a delayed floral organ abscission mutant in Arabidopsis thaliana

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The process of abscission, the shedding of organ systems such as leaves, fruits, and flowers, is a common phenomenon in the course of normal development of higher plants, yet the physiological and genetic basis of abscission is still not entirely clear. What is evident is that abscission is an active, highly regulated and complex process that occurs at defined zones of anatomically distinct cells (the abscission zone) that undergo enlargement and subsequent cell separation. The use of mutants and transgenic plants in Arabidopsis provide important tools in elucidating the series of molecular events that culminate in abscission, and the Patterson lab has identified several mutants delayed in the abscission of floral organs in T-DNA insertion lines (University of Wisconsin T-DNA populations). One of these mutants, *dab5*(delayed abscission 5), retains petals past position 17 on the inflorescence, whereas abscission of petals normally occurs at position 6 in WS wildtype plants. Scanning EM photos of the abscission zone of *dab5* mutants indicate that cells in this region undergo uncontrolled elongation rather than forming rounded cells like wildtype. Southern blot analysis has indicated the presence of a single T-DNA insertion within the genome of *dab5* plants that cosegregates with the mutant phenotype, and work is ongoing to confirm that this insertion is responsible for the delayed abscission phenotype. This poster will present a detailed analysis of the phenotypic and genetic characterization of *dab5*, including scanning EM, petal breakstrength measurements, and preliminary molecular data.This work was supported by USDA Grant 0035301-9085 and ATG training Grant NSF/DOE/USDA DB1 960-2222

207 Quantitating expression of individual actin gene family members in single Arabidopsis cells.

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We have developed a novel technique, single cell RT-PCR, that allows us to detect and quantitate individual gene transcripts in single Arabidopsis cells. As a proof-of-principle study we have applied the technique to measure the differential expression of the genes encoding eight different actin isoforms. Under a microscope, the cell contents were extracted from individual epidermal or mesophyll cells using glass micro-capillaries, and from trichomes by excision. The levels of the individual mRNAs were measured by semi-quantitative RT-PCR, using isoform-specific gene primers. To exclude the possibility that extracts from epidermal cells had been contaminated by the contents of mesophyll cell, we also assayed for the presence of *rbcS*. In most of the epidermal samples we were unable to detect any *rbcS* mRNA, indicating that levels of contamination if any, were very low. In both mesophyll and epidermal cells we consistently detected expression of *ACT2* and in about half of the samples we also detected *ACT8*. In trichomes we consistently detected both *ACT2* and *ACT8* and in addition, transcripts of *ACT7* and *ACT11*. We never detected expression of any of the other actin isoforms. This work confirms and extends the spatial resolution of expression previously reported for actin genes and demonstrates the practicability of the technique. Most significantly, single cell RT-PCR allows gene expression to be quantitated at the ultimate biological resolution, that of the individual cell. We are now extending this technique to the analysis of changes in gene expression in Arabidopsis cells following infection by a virus.

208 Identification, characterization and subcellular targeting of prenyl and acyl lipids-modified proteins

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Prenylation and acylation are posttranslational protein modifications required for the activity and subcellular localization of several proteins, which function in signaling cascades that regulate growth, development and stress. Regardless of their importance, the role of these lipid modifications in plant signaling is not well understood. An experimental scheme was developed that combines extensive database searches, together with robust utilization of Arabidopsis and yeast mutants and biochemical analysis. So far, these studies have yielded the identification of a number of prenylated and acylated proteins and information about preferable protein substrates and their subcellular targeting pathways. Prenylation by farnesyltransferase (FTase) and geranylgeranyltransferase-I (GGTase-I) involves cysteine residues, which are part of a conserved C-terminal CXXX sequence motifs commonly known as CaaX box. Our studies show that Ca_1a_2X proteins in which the a_2 position is occupied by charged amino acids are poor substrates of either FTase or GGTase-I. On the other hand, a polybasic domain proximal to the CaaX box induced a five-fold increase in V_{max} and an order of magnitude decrease in K_m for prenylation by GGTase-I. These data indicate that most substrates of GGTase-I may contain a polybasic domain or alternatively should be expressed at higher levels. Following prenylation proteins undergo two additional processing events at their C-terminal end. The aaX moiety is removed and in turn the free carboxyl group of the isoprenyl cysteine is methylated. We show that prenylation occurs in the cytoplasm while aaX processing and carboxy-methylation occur in the ER and golgi membranes, respectively. These data suggest that prenylated proteins are targeted to their location through the endomembrane system. Several proteins, however, are targeted by a different mechanism. Examples will be shown of proteins, which are acylated or prenylated and acylated. Targeting of these proteins is defined via a balance between prenylation and yet unidentified factors.

209 Effects of wounding on RNase induction in *Arabidopsis thaliana*: *RNS1* defines a novel signaling pathway

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Induction of defense-related genes is one way plants respond to mechanical injury. In Arabidopsis, it has been shown that at least two pathways control gene expression in response to wounding: jasmonic acid (JA) -dependent and -independent. A current model suggests that JA is responsible for transcripts that accumulate in non-damaged, systemic tissue, and a second factor, oligogalacturonides, controls gene expression in local damaged tissue. Because ribonuclease (RNase) activities have been shown to be induced in other systems in response to wounding or pathogen challenge, we investigated whether RNases are involved in the wounding response in Arabidopsis. In damaged leaves, stems, and seedlings, several RNases are induced with various timings. Activities induced include at least three bifunctional nucleases capable of degrading both RNA and DNA. Additionally, the activity of RNS1, a member of the widespread RNase T2 family, is strongly induced in response to wounding, and the *RNS1* transcript is induced both locally and systemically. However, RNS1 accumulation is not controlled by JA. In fact, all three nuclease activities, as well as RNS1, are induced by wounding in the JA-insensitive *coi1* mutant, demonstrating that the induction of all the activities is independent of JA-signaling. Thus, we have demonstrated that a JA-independent wound-responsive gene can be induced systemically. Further, induction of these activities is not controlled by oligosaccharide elicitors. Consequently, a novel pathway, likely involving a third signal, exists in Arabidopsis. This hypothesis, as well as further studies of RNase expression and possible functions in wounded plants, will be discussed.

210 An *Arabidopsis* sec13 homolog that interacts with ADL6 functions as a negative regulator of trafficking from the TGN to the central vacuole

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Large number of proteins are transported from the trans-Golgi network to the central vacuole after translation. However, the mechanism of the trafficking is not well understood in plant cells. We isolated a cDNA, named Seh1 homolog (Seh1h) by a yeast two hybrid screening using C-terminal region (356 amino acid residues) of *Arabidopsis* dynamin-like 6 (ADL6) as a bait. The cDNA encoded 326 amino acid residues with a calculated molecular weight of 35.8 kDa. The deduced amino acid sequence showed about 20% amino acid sequence identity to Seh1 and yeast Sec13. Protein-protein interaction between Seh1h and ADL6 was confirmed by the *in vitro* pull down assay using recombinant ADL6 and Seh1h proteins expressed in *E.coli*. Also, an immunoprecipitation experiment revealed that a polyclonal anti-Seh1h antibody precipitated both Seh1h and ADL6 from protein extracts prepared from *Arabidopsis* leaf tissues. The biological role of Seh1h was addressed *in vivo* using an *in vivo* trafficking assay using GFP:EBD and sporamin:GFP as cargos for the transport from the TGN to the central vacuole. The *in vivo* assay showed that Seh1h-dN accelerated transport of GFP:EBD and sporamin:GFP to the central vacuole. Taken together we proposed that Seh1h functions as a negative regulator of vacuolar trafficking from the TGN.

211 A short hydrophilic region located at the C-terminus of the transmembrane domain is required for targeting of AtOEP7 to the outer envelope membrane of chloroplast.

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The small outer envelope membrane proteins of chloroplasts are synthesized at their mature size in the cytosol without a cleavable N-terminal transit peptide. Here, we investigated the chloroplastic outer membrane targeting signal of AtOEP7, an *Arabidopsis* homolog of the small outer envelope membrane proteins, *in vivo*. AtOEP7 was expressed as fusion protein with green fluorescent protein(GFP) either transiently in protoplasts or stably in transgenic plants. In either case, flourescence microscopy of transformed cells and western blot analysis of fractionated proteins confirmed that the AtOEP7:GFP fusion protein was targeted to the chloroplast outer envelope membrane. Experiments using various amino acid substitution and deletion mutants revealed that a short hydrophilic region located at the C-terminus of the transmembrane domain is required for targeting of AtOEP7 to the chloroplast. Also, the transmembrane domain has preference for amino acid residues with small side chains for insertion into the chloroplast outer envelope membrane. In addition, a fusion protein, AtOEP7:NLS:GFP, was efficiently targeted to the chloroplast outer envelope membrane despite the presence of the nuclear localization signal, suggesting that the protein may be associated with a cytosolic factor during its translocation to the chloroplast outer envelope membrane.

212 Characterization of Gain-of-Function *bri1* Suppressors

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Cell surface receptor-like protein kinases (RLKs) play fundamental roles in regulating plant growth and development. Our goal is to understand how extracellular signals are perceived and transduced through RLKs. Brassinoteroid-insensitive 1 (BRI1) encodes an RLK which is required for the perception of brassinosteroids. We carried out a gain-of-function genetic screen with a weak *bri1* allele, *bri1-5*. We screened over 40,000 *bri1-5* activation tagging lines and identified twelve suppressors. Several of these suppressors have been cloned and one suppressor, *brs1-1D*, for *bri1 suppressor-dominant 1*, has been characterized in detail. *BRS1* encodes a putative secreted serine carboxypeptidase. The suppression requires a functional BRI1 kinase domain, brassinosteroids and BRS1 enzyme activity. Neither overexpression of *BRS1* in wild-type nor *brs1* loss-of-function lines show any obvious phenotype. There are at least 5 closely related BRS1 genes in *Arabidopsis* which share 50-70% protein sequence identity with BRS1. At least one of these homologs can also suppress *bri1-5* upon overexpression. We hypothesize that BRS1 and homolog(s) regulate an early event in BRI1 signaling, either processing an unidentified steroid-binding protein or BRI1 itself. Two other *bri1-5* suppressors, which resemble the suppression phenotype seen in *brs1-1D*, have also been characterized. In both lines, the *BRI1* locus was activation tagged. Together, these results suggest that the *bri1-5* mutation causes BRI1 signaling to be rate-limiting, which can be overcome by either increasing the amount of BRS1 or the BRI1 receptor itself.

213 Membrane association of calcium-dependent protein kinases (CDPKs) in Arabidopsis

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The calcium-dependent protein kinase (CDPK) gene family in Arabidopsis contains 34 members. Twenty-nine of the predicted CDPK proteins have myristoylation consensus sequences at their amino termini. Modification of proteins by myristate, a 14-carbon fatty acid, is known to increase membrane binding. Myristate is linked to proteins via an amino-terminal glycine residue if that glycine is in the context of a short consensus motif. We are investigating CDPK isoforms CPK2, CPK5, and CPK6 and have shown that these three proteins are at least partially membrane associated in planta. We are studying both the subcellular membrane location of each of these isoforms, as well as the role that myristoylation plays in membrane association. Subcellular localization studies were conducted using sucrose density gradient fractionation of plant microsomes. Markers for different membrane types were detected by a combination of western blotting and enzyme assays. CPK2 appears to be associated with the endoplasmic reticulum, while CPK5 and CPK6 are not. The exact subcellular locations of CPK5 and CPK6 are currently under investigation. The CPK2 isoform is tightly bound to the membrane fraction and was only removed by treatment with detergents. A coupled transcription/ translation system was used to study the myristoylation of CDPK isoforms in vitro. Mutation of the glycine at the site of myristate attachment is known to prevent myristoylation. We have used site-directed mutagenesis to create CDPKs that can not be myristoylated in vitro and then investigated the membrane association of these proteins in planta. Our results indicate that different CDPK isoforms are targeted to different subcellular membrane locations and that myristoylation is important in membrane association.

214 The *TARDY ASYNCHRONOUS MEIOSIS*(*TAM*) gene is required for the normal pace and synchrony of cell division during *Arabidopsis* male meiosis

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Male meiosis in higher organisms features synchronous cell divisions in a large number of cells. It is not clear how this synchrony is achieved, nor is it known whether the synchrony is linked to the regulation of cell cycle progression. In the process of screening EMS-mutagenized populations for mutations affecting *Arabidopsis* pollen development, we isolated a mutant, named *tardy asynchronous meiosis* (*tam*), that exhibited a phenotype of delayed and asynchronous cell divisions during male meiosis. In *Arabidopsis*, two nuclear divisions occur before simultaneous cytokinesis yields a tetrad of haploid cells. In *tam*, cell divisions are delayed, resulting in the formation of abnormal intermediates, most frequently dyad meiotic products, or in rare cases, dyad pollen (two gametophytes within one exine wall). Analysis of *tam* and the *tam*/*qrt* double mutant showed that these abnormal intermediates could continue through the normal rounds of cell divisions and form some functional pollen, though at a slower than normal pace. The asynchrony of cell division started at the G2/M transition, with nuclei entering mitotic phase at different time points, during both meiosis I and meiosis II. Chromosome mis-segregation sometimes occurred, leading to the formation of extra, smaller spores and/or multiple nuclei in one cell. Temperature-shift experiments showed that the phenotype was more severe at 27 °C than at 22 °C. These observations suggest that the TAM protein positively regulates cell cycle progression, perhaps by promoting the G2/M transition, and that the normal pace of cell cycle progression might be coupled with the synchrony of cell division during male meiosis. We speculate that the cyclin B/Cdk1 pathway, known to regulate the G2/M transition, might respond to a signal that synchronizes cell division.

215 Chimeric repressor interference: A novel method for the analysis of plant transcription factors

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Transcriptional control presently seems to be the most important level of gene regulation in eukaryotes. Different types of signals, e.g. intra- or intercellular communication, physiological feedback, and environmental inputs are integrated to cell-specific transcription levels for each gene. To gain access to underlying plant regulatory networks, we have developed a new tool, allowing analysis of transcription factors *in planta*. In the *Chimeric Repressor Interference System (CHRIS)* plant transcription factors are converted to dominant-negative repressors. After expression in transgenic plants the chimeric proteins may displace the native gene product from target sites or titrate auxiliary proteins, resulting in trans-dominant phenocopies of loss-of-function alleles. Thus *CHRIS* provides a rapid method to elaborate the biological function of transcription factors identified in genomic sequence data. Acting on the protein level *CHRIS* should be informative in redundant situations and suitable to transfer knowledge from model to crop species.

CHRIS was used exemplarily on the gene *SHOOT MERISTEMLESS (STM)*, encoding a homeodomain-type transcription factor, which is expressed in the shoot apical meristem. Expression of the chimeric *STM* protein in transgenic plants resulted in a phenocopy of the *stm* loss-of-function phenotype. Additionally an inducible dominant-negative GR-fusion was created, which allows to block meristem activity after induction with dexamethasone, demonstrating that *CHRIS* is working on the protein level. A careful deletional analysis of the *STM* gene product by use of *CHRIS* allowed the association of individual protein domains to different phenotypes, which are best explained by protein-protein interactions. Potential interacting protein partners have been identified in the yeast-two hybrid system and belong to various families of transcription factors. *CHRIS* was also used on the *KNOTTED1*-like homeodomain transcription factor *KNAT1*. Although a loss-of-function phenotype of *KNAT1* is not known so far, the *CHRIS* technology indicates a major function in the inflorescence.

216 The *fat root*gene is responsible for the cortical microtubule alignment during the directional cell elongation in *Arabidopsis*.

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Extensions of axial plant organs such as stems and roots are caused by elongation of individual cells in the organs. The determination of the axis of cell expansion is an essential process in the morphogenesis of plant organs. The cortical microtubule array is known to influence the direction of cell expansion by providing spatial templates for cellulose microfibril deposition. Thus, the interphase array of microtubules plays an important role in the morphogenesis of plant cell. But little is known about the factors, which regulate the formation of interphase cortical array.

We reported a mutation, *fat root* (*ftr*), which affects an axis determination of the cell expansion in all cell types. Mutant seedlings displayed abnormal increase of root diameter caused by irregular lateral cell expansion. In the *ftr* mutant, cortical microtubules exhibit more random alignment rather than that of wild type. It suggests that the FTR protein participates in construction of the cortical microtubule array.

We have cloned the *FTR* gene using a map-based cloning strategy. The *FTR* gene encodes a katanin-like protein, which is known as a microtubule-severing protein. To identify the function of FTR protein, we raised antibodies against the recombinant FTR proteins. The anti-FTR antibody recognized single band on SDS-PAGE gel at approximately 60kDa. We confirmed the expression of the *FTR* gene by Northern and Western blot analyses of various organs and found a predominant expression of *FTR* in inflorescences and roots, with ubiquitous lower expressions in all other organs. Since our inflorescence and root samples contain shoot and root apical meristems, such higher expression in these organs strongly suggests that the FTR protein may function immediately after the cell division, which is a time point of cortical microtubules reconstruction. These results suggest that the FTR protein can interact with the microtubules during interphase and can control the stability of cortical microtubules. Progress in the cellular localization analysis of FTR protein will be presented.

217 Role of ATHB-2 in plant development

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The Arabidopsis *ATHB-2*gene encoding a HD-Zip protein is rapidly and strongly induced by changes in the red to far-red light ratio which naturally occur during the day under the canopy and induce the shade avoidance response. Analysis of transgenic seedlings bearing constructs that alter ATHB-2 expression revealed a series of developmental phenotypes. Seedlings overproducing ATHB-2 had longer hypocotyls and petioles, and smaller cotyledons. Moreover, these seedlings also had a thinner root mass than controls. Conversely, seedlings with reduced levels of ATHB-2 had shorter hypocotyls, larger cotyledons, and a thicker root mass than wild type. Together with the tight regulation of *ATHB-2* by the phytochrome system, these data imply a major role for this HD-Zip protein in the regulation of the shade avoidance response. However, the antisense phenotype strongly suggests that ATHB-2 plays a regulatory role also under normal growth conditions. To gain more information on ATHB-2 function, a collection of 8,000 plants carrying, on average, six independent insertions of the En-1 element was used for reverse genetic analyses to identify knock-out alleles of *ATHB-2*. Two insertions in the *ATHB-2*coding sequence, named 6N13 (*athb2-1*) and 6AAB113 (*athb2-2*) were identified. Northern blot analyses showed that *ATHB-2*mRNA is below the level of detection in the two mutants. To further investigate ATHB-2 function, transgenic plants expressing a derivative of ATHB-2 containing a single aa substitution in the HD (ATHB-2N51A) which is known to abolish DNA binding of homodimeric as well as heterodimeric complexes were also generated. This mutation is expected to produce a dominant-negative phenotype. Indeed, at the seedling stage, the phenotype of the ATHB-2N51A lines is the same as that of the antisense lines, but more severe. At later stages of development, the dominant-negative plants produce more leaves and a higher number of lateral inflorescences than the controls. Conversely, plants overproducing ATHB-2 had fewer leaves and a reduced number of lateral inflorescences. The phenotypic characterization of *athb-2*and ATHB-2N51A plants is in progress.

218 Functional analysis of the 37-kDa inner envelope membrane polypeptide in chloroplast biogenesis, using a Ds-tagged Arabidopsis pale green mutant.

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To study functions of nuclear genes involved in chloroplast development, we systematically analyze albino and pale green *Arabidopsis thaliana* mutants using a two-component transposon system based on the *Ac/Ds* element of maize as a mutagen. One of the pale green mutants, designated *apg1* (for albino and pale green mutant 1), could not survive beyond the seedling stage when germinated on soil. The chloroplasts of the *apg1* plants had decreased lamella with reduced levels of chlorophyll. A gene encoding a 37-kDa chloroplast inner envelope membrane polypeptide precursor was disrupted by an insertion of transposon in *apg1*; the 37-kDa protein had partial sequence similarity to S-adenosylmethionine dependent methyltransferase. The *apg1* plants lacked plastquinone suggesting that APG1 protein is involved in the methylation step of plastoquinone biosynthesis that localized at the envelope membrane. Present study demonstrates the importance of 37-kDa chloroplast inner envelope membrane protein for the chloroplast development in *Arabidopsis*.

219 Membrane dynamics during cell morphogenesis in Arabidopsis

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Differentiation of cells into specialized tissues is a hallmark of multicellular organisms, and results in generation of a recognizable external appearance in both plants and animals. While much study has focused on how signals are generated that direct body plan formation in these organisms, little is known about mechanisms controlling reorganization of cellular architecture within single cells. Ultimately, dynamic changes within single cells are required for altered cell morphologies observed in the tissues of multicellular organisms. In plants, cell walls and a large central vacuole, fundamentally influence development. Cell walls hinder cell expansion, and impede changes in cell shape. Because of this, increase of vacuole size is required for cellular expansion, and often accompanies changes in cell morphology. Therefore, understanding how vacuolar biogenesis and positioning are regulated, and how cell wall proteins and hemicelluloses are delivered to specifically to plasma membrane regions of cell wall expansion are key events in understanding how cell morphology is altered during differentiation. Several lines of evidence now implicate links between Rab GTPases (a protein family that regulates membrane trafficking events) and attachment of organelles to, or movement along, the cytoskeleton in animal cells. Because membrane trafficking events are essential for both vacuole biogenesis and delivery of cell wall components to the plasma membrane it is likely that Rab GTPases play a role in these processes. Additionally, organelle positioning within cells and orientation of trafficking pathways rely on cytoskeletal interactions, further implicating potential roles for Rab GTPases within plant cells undergoing changes in morphology. To test this, we have cloned several plant Rab GTPases predicted to regulate post-Golgi trafficking pathways and are investigating their possible roles in cell differentiation in Arabidopsis using a combination of time-lapse video microscopy, biochemistry, and cell biology.

220 TAO1 is required for susceptible host responses to the Pseudomonas syringae type III effector AvrB

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Most plant pathogenic bacteria use type III secretion systems to deploy effector proteins into the host cell. Genetic analysis has identified Avr proteins as effectors of the type III secretion system in phytopathogenic bacteria. On resistant plants, Avr proteins are elicitors of defense responses via the activation of appropriate Resistance proteins. In contrast, many Avr proteins are known to contribute to virulence on susceptible host plants. The mechanisms by which Avr proteins exert their effects in either case are not clear. We would like to identify components and pathways which are targeted by Avr proteins during infection on susceptible hosts. We have taken a genetic approach to identify targets of the *Pseudomonas syringae* type III effector AvrB on susceptible *Arabidopsis* ecotypes. AvrB induces a chlorotic response when expressed in susceptible ecotypes. Using a conditional expression system, we have identified *Arabidopsis* mutants which fail to exhibit AvrB-induced chlorosis. The *Target of AvrB Operation1* (*TAO1*) mutant may help illuminate how bacteria cause disease in susceptible hosts. In addition, *tao1* mutants may also help elucidate how Resistance proteins recognize Avr proteins. Data will be presented on the characterization of *tao1* interactions with pathogens and possible connections to components of the Rpm1-AvrB recognition complex.

Rpm1 work in the Dangl Lab is supported by DOE grant DE-FG05-95ER20187 to JLD. D.M. is a DOE Fellow of the Life Sciences Research Foundation

221 Analysis of component of the plastid protein import apparatus in Arabidopsis

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Nuclear-encoded chloroplast precursor proteins are imported into chloroplast by preprotein translocases, which are located both in the outer and inner envelope membranes of the organelle. The translocon at the outer membrane of chloroplasts (Toc complex) and the translocon at the inner membrane of chloroplasts (Tic complex) act co-operatively during the import process. Although putative components of the import apparatus have been identified biochemically, their role in import remains to be proven in vivo. Arabidopsis mutants lacking a component of the import machinery has been isolated. In vivo role of the translocon component in plastid protein import will be discussed.

222 Multidrug Resistance-Like Genes Required for Auxin Transport and Auxin-Mediated Development

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The *multidrug resistance* (*MDR*) genes in animals encode transporters that enhance the efflux of hydrophobic drugs from the cytoplasm of tumor cells. Arabidopsis possesses six *MDR*-like genes, one of which, *AtMDR1*, was shown to be induced by the hormone auxin. Mutants in *AtMDR1* and *AtPGP1*, the next most closely related gene in Arabidopsis, were obtained by screening T-DNA mutagenized populations of plants. Epinasty of the cotyledons and first leaves, resembling auxin-treated wild-type seedlings, was the most obvious phenotype of *atmdr1* knock-out seedlings. The inflorescence of adult *atmdr1* plants grew more slowly but otherwise the mutant was similar to wild type. Transformation of *atmdr1* plants with the wild-type gene complemented all aspects of the phenotype. No obvious phenotypes were observed in juvenile or adult *atpgp1* plants. However, double mutants lacking both *MDR*-like genes displayed greater epinasty and curled leaves. Apparently AtMDR1 can compensate for the loss of AtPGP1, but the reverse is not true. Adult double mutants grew more slowly and were much more highly branched than wild type, indicating that these two genes control apical dominance. Measurements using radioactive IAA revealed that basipetal auxin transport in hypocotyls and inflorescence stems was impaired by the *atmdr1* mutation, and almost abolished in the double mutant. Yeast expressing AtMDR1 were used in studies of AtMDR1 function. No evidence of IAA transport in these yeast has yet been obtained but NPA, the chemical inhibitor of polar auxin transport, bound tightly and specifically only to AtMDR1-expressing yeast. Remarkably, an independent line of biochemical research identified AtMDR1 and AtPGP1 as plasma membrane proteins that could be purified from Arabidopsis preparations by NPA-affinity chromatography. The results indicate that these two *MDR*-like genes of Arabidopsis encode NPA-binding proteins that are required for normal auxin distribution and auxin-mediated genesis of plant form.

223 Tomato as a model system to understand the compatible response in Arabidopsis.

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A plant's capacity to synthesize or react to ethylene, jasmonic acid (JA) or salicylic acid (SA) is known to affect any subsequent response of the plant to attempted pathogen invasion. To date, the relative importance of each of these stress signals has focused on their role in the incompatible response, while their action in the compatible response of plants, has been less well studied. We have undertaken a series of experiments in tomato, in which we have addressed some of the fundamental questions related to a plant's response to virulent, disease causing, pathogens. In these experiments tomato lines with altered ethylene, JA or SA metabolism were infected with Xanthomonas campestris pv. vesicatoria (Xcv). In all the lines tested the loss of ethylene JA or SA signaling was found to neither inhibit nor promote bacterial growth, yet the level of disease in all these lines was visibly reduced, compared to that in their wild-type controls. We have termed this pseudo-resistant response "tolerance" as the marked reduction in tissue necrosis was not associated with an inhibition in pathogen growth. SA analysis in each of the tested lines show that its accumulation is a late event in the compatible response, coincident with the massive necrosis observed in the wild-type susceptible lines, but absent in the tolerant lines compromised in ethylene and/or JA signaling. SA accumulation in tomato in response to a virulent strain of XCV is therefore dependent upon a prior action of the plant to both ethylene and/or JA. To further understand this possible relationship between ethylene action and SA accumulation, we have decided to take advantage of the numerous Arabidopsis ethylene signaling mutants. We are currently assessing the level of disease and SA accumulation in etr1,etr2 ers1, ers2, ein4 and ein2 in response to Xanthomonas campestris pv. campestris (Xcc) and Pseudomonas syringae pv. tomato (Pst). The data we will present will focus on three main areas: SA accumulation following infection; how this relates to the differences in disease symptom production in the various ethylene signaling mutants and finally, how together these results suggest that Arabidopsis and tomato appear to regulate the compatible response in a cultivar specific manner.

224 Functional analysis of Arabidopsis Response Regulators, ATRR1/ARR4/IBC7 and ATRR3/ARR8 in transgenic plants.

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Arabidopsis *ATRR1*/*ARR4*/*IBC7* and *ATRR3*/*ARR8* are homologous genes of prokaryotic response regulators that are involved in the His-Asp phosphorelay signal transduction. It has been shown that the expression of the *ATRR1*/*ARR4*/*IBC7* gene is induced by cytokinin, while the response to the cytokinin of the *ATRR3*/*ARR8* gene expression is slightly lower than that of *ATRR1*/*ARR4*/*IBC7* gene. In the present study, we analyzed the function of these two genes as response regulators using transgenic plants. Overexpression of ATRR1 in cultured stems of the transgenic plants allowed to form shoots abundantly in the presence of cytokinin, while over-expression of ATRR3 repressed shoot formation and greening of calli. The expression level of cytokinin-inducible genes, *cycD3* and *cab* increased in the ATRR1 overexpresser but decreased in the ATRR3 overexpresser. In contrast, the expression levels of two drought stress-inducible genes, *rd29A* and *erd1* in both transgenic plants were in the similar level as those in control plants. These results suggest the possibility that ATRR1 and ATRR3 are involved in cytokinin signal transduction, and that the ATRR1 functions as a positive-regulator, whereas the ATRR3 functions as a negative-regulator, or that the ectopic expression of ATRR3 which may not be involved in the cytokinin signaling causes a negative effect.

225 Enhancer trap lines with GUS expression in developing Arabidopsis fruits

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The *Arabidopsis* fruit is representative of fruits from >3000 plant species belonging to the *Brassicaceae* family. It mediates seed maturation and eventually springs open dispersing the seeds in a process called pod shatter, or fruit dehiscence. The fruit is a highly specialized organ and serves a unique system for studying cell differentiation with establishment of distinct cell types closely positioned in rows along the fruit. Although the early regulators of dehiscence zone formation have recently been identified, little is konwn about the subsequent cascade of gene activity that leads to pod shatter. As a start toward identifying downstream genes involved in this process, we have used an enhancer trap screen approach and have identified several lines with GUS-expression patterning different cell types of the fruit (see also poster by Roeder et al.). In one of these (YJ80), GUS-staining appears at the valve margin and in the abscission zone at the seed attachment site on the funiculus. Genetic analysis positions YJ80 GUS-expression downstream of the MADS box genes, *SHATTERPROOF1* and *2* (*SHP1/2*) and of *INDEHISCENT1* (*IND1*) - encoding a bHLH protein. The T-DNA is inserted ~2kb upstream of a gene encoding a protein of unknown function, but with weak similarity to mammalian Ankyrins. This gene belongs to a family of 29 highly similar genes in Arabidopsis – all encoding putatively membrane-bound proteins. Another line (YJ8) has GUS-expression in 3 to 4 cells of the valve margin inner epidermis and at the sepal and petal abscission zones. YJ8 GUS-expression appears to be positively regulated by *SHP1/2*. The T-DNA is inserted ~3 kb upstream of a gene encoding a Xyloglucan endotransglycosylase related (XTR) protein. Cell wall modifying enzymes are known to participate in the fruit maturation process and the XTR gene thus seems a likely candidate for being one of probably several targets downstream in the cascade. Data on loss-of-function mutants and gain-of-function transgenes will be presented.

226 The ozone sensitive *rcd1* mutant: a system for studying the regulation of radical induced programmed cell death

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The gaseous pollutant ozone (O_3) has become established as a simple and effective way of applying reactive oxygen species (ROS) in order to study the genetics of ROS signaling. We have previously described the isolation of the *rcd1* (radical induced cell-death) mutant. *rcd1* is a codominant single locus trait on ch 1 (at ca. 51.4cM) and confers hypersensitivity to O_3, superoxide, avirulent pathogen challenge, but not hydrogen peroxide. In addition to its increased initial level of cell death *rcd1* also has a transient spreading cell death phenotype that continues until ca. 1 d. post challenge. We have shown that this superoxide-driven spreading cell death is ethylene dependent and can be counteracted by jasmonate. The map based cloning of *rcd1* is underway. O_3 exposed plants typically display markers associated with the hypersensitive response (HR). It is commonly held that O_3 induces an HR-like cell death program. This strongly implies that O_3-induced cell death is programmatic in nature; although this fact has never been shown directly. To address this we have explored cell death in *rcd1* facing various ROS challenges. We show that O_3 exposed *rcd1* exhibits hallmark characteristics of programmed cell death including nuclear shrinkage and fragmentation, cell shrinkage, chromatin condensation, and nuclear DNA fragmentation. Furthermore, cell death induced by ROS challenge can be reduced or delayed with inhibitors of active metabolism and also serine-protease- and caspase-inhibitors. The highly controlled O_3-induced biosynthesis of signal molecules such as salicylate, jasmonate, and ethylene provides further evidence of the similarities between biotic and O_3 pathologies. The interaction and balance between these signals is involved in cell death regulation. The further use of hormones, pharmacological-elicitors and -inhibitors to dissect some of the early radical induced signal transduction events leading to stress ethylene evolution and cell death will be discussed.

227 A Novel Screen for the Isolation and Characterisation of New Circadian Clock Mutants in Arabidopsis thaliana.

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The hypocotyl of wild-type Arabidopsis seedlings is significantly elongated when plants are grown under short photoperiods (8L:16D, the pattern of 8 hours of light and 16 hours of darkness), as compared to plants grown under constant light. This elongation under short days was accentuated in the circadian-clock mutants *early flowering 3* (*elf3*), and *late elongated hypocotyl* (*lhy*), and in transgenic plants that overexpressed *circadian clock associated 1* (*cca1-ox*). All three mutants showed a wild-type hypocotyl under constant light, but a significantly longer than wild-type hypocotyl under 8L:16D.

This elongated phenotype was used as the basis for a primary screen, towards the isolation of novel circadian clock mutations. We screened T-DNA mutagenised seed populations obtained from the Nottingham Arabidopsis Stock Centre, including lines from the INRA Versailles and Feldman labs, and tagged-activation lines from the Weigel lab. Mutants with confirmed hypocotyl phenotypes were taken through a secondary screen, involving the characterisation of their circadian rhythms of leaf movements.

From a total of approximately 14,000 lines (screened in pools of 20 or 100), 4 long period and 5 short period mutants were identified. One mutant, *slowcoach* (*slo*), has been chosen for further characterisation. The period of leaf movement rhythms is lengthened in slo (26.7 hours, as compared to 23.7 hours for wild-type plants). It also displays altered photoperiod responsiveness, *slo* plants flower earlier than wild type under both short- and long-day photoperiods.

Current work on *slo* includes examination of the expression of various rhythmic genes using luciferase reporter fusions, mapping of the mutation and cloning of sequences flanking the T-DNA.

228 Investigation of Plant Cell Wall Biosynthesis in Arabidopsis Using Xyloglucan Fucosyltransferase

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Plant cell wall biosynthesis is a process critical for plant growth, development, and defense against pathogens. In order to better understand how this process is regulated, the cell wall biosynthetic gene *AtFT1* encoding xyloglucan fucosyltransferase (XyG FucT) has been studied throughout tissues of wild type Arabidopsis. XyG is the major hemicellulose of dicotyledonous plants, and is composed of a glucan backbone with side chains of xylose alone, xylose and galactose, or xylose, galactose, and fucose. This polymer is thought to interact with cellulose microfibrils and thus affect structural integrity of the cell wall. Promoter-reporter studies have been conducted so that *AtFT1* gene regulation in various regions of the plant may be visualized. Additionally, a panel of Arabidopsis tissues has been analyzed to determine 1) levels of *AtFT1* gene expression using quantitative RT-PCR, 2) levels of XyG FucT activity in Golgi vesicles prepared from these tissues, and 3) the extent of fucosylation of XyG as determined by NMR and MS analysis. The goal of these studies is to determine whether XyG structure varies in different tissues of Arabidopsis, whether regulation of XyG FucT may occur, and whether such regulation correlates with growth (and thus cell wall deposition) and/or structural variation. Preliminary data indicate that differences in amount of XyG FucT activity levels do occur in various tissues and correlate with regions of active growth. Expression analysis is ongoing to determine whether regulation occurs at the transcriptional level. XyG fucosylation appears to be consistent throughout Arabidopsis tissues with the exception of rosette leaves, which appear to contain approximately half the XyG fucose residues found elsewhere in the plant.

229 The *Arabidopsis thaliana* RHA1 gene encodes a new heath shock factor, a possible transducer of signals coming from auxin and gravity in plant roots

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The mutant *rha1* shows in the roots reduced gravitropic response, and reduced slanting toward the right-hand, together with increased resistance to the auxinic hormones, their inhibitors, and to ethylene. Taking advantage of a T-DNA tag inserted in RHA1, we isolated a fragment of the gene from the T-DNA left border, and used it to identify its DNA sequences from the TAIR DataBank, and to get through RT-PCR its cDNA. The gene appears to be a new heat shock factor (HSF), made up of two ORF and a 164 bp intron in between. The gene maps on chromosome 5, close and above the RFLP marker mi61. RT-PCR confirmed, as expected, that the gene is expressed in the wild-type, but not in the mutant. Complementation of RHA1 is presently carried out through *A. tumefaciens* transformation. RHA1 shows notable homology in the DNA binding motiv, at the level however of the aminoacidic sequences, with other HSFs from plants (*Arabidopsis*, tomato and maize), *Cenorhabdites*, Drosophila, mouse, yeast, and humans. By contrast, the rest of the DNA sequences show no apparent homology with other HSFs. In addition, RHA1 is lacking of the terminal hydrophobic repeat HR-C, generally present in other HSFs, but not in HSF4 from humans, and in a HSF from budding yeasts. We hypothesize that RHA1, apart from its function in the activation of HSP, could be involved in the transduction of signals coming from gravity and auxin. It could thus be one of the sought elements of the gravitropic signal transduction pathway of plant roots. Possibly, as shown for the HSF2 from humans (Hong Y. and Sargent K.D., 1999 J. Biol. Chem. 274, 12967- 70), it could be involved in the regulation of the PP2A phosphatase, which has been demonstrated to regulate the trasport of auxin in *Arabidopsis* (Garbers C. et al. 1996 EMBO J. 15, 2115-2124). We plan soon to start investigating RHA1 functions, through in situ hybridization and reporter genes.

230 The *Multiple Response Expansion* Genes and Root Cell Shape in *Arabidopsis thaliana*

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Plant form is determined by the shapes and sizes of the component cells, yet remarkably little is known about the molecular mechanisms by which plant cell shape is determined. We have identified a novel class of mutants in which cell shape is altered in the roots of the model plant *Arabidopsis thaliana*. The *multiple response expansion* mutants (*mre*s) have roots that are significantly shorter and thicker than wild-type, similar to the root cell shape mutants that are affected in cell wall synthesis (*radial swollen1*, *korrigan*, and *procuste*). The *mre*s are distinct, however, in that their phenotypes are dependent not only upon growth conditions, but also upon ethylene perception and responses. The three *MRE* loci identified to date have been mapped to novel locations on chromosomes 1 and 5. We are currently fine mapping *mre1* with the intent of cloning the *MRE1* gene.

231 The *SPIKE* gene is essential for epidermal cell development

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The cytoskeleton regulates cell polarity and shape during differentiation. By screening for mutations that have specific effects on trichome morphogenesis we have identified several genes that may be directly involved in the organization of the actin or microtubule cytoskeletons. To identify essential genes in the cytoskeletal organization pathway we have screened for seedling lethal mutants that also have specific trichome shape defects that are able to be phenocopied by microtubule or F-actin-disrupting agents. The *spike* mutation affects trichome branch growth, epidermal pavement cell morphogenesis, and tissue organization. We have isolated three *spike* alleles and have phenocopied the *spike* phenotype by introducing double-stranded RNA corresponding to small regions of the *SPIKE* gene into wild-type plants. The *SPIKE* gene has been cloned, and encodes an 1830 amino acid protein that shares extensive amino acid identity with a number of RAC-binding proteins from rat, humans, flies, and worms. In animal cells these proteins are hypothesized to reorganize the actin cytoskeleton at integrin-containing complexes. The hypothesis that SPIKE integrates extracellular signals and cytoskeletal re-organization is a major research question in our lab. We will present a detailed analysis of the gene expression pattern, the *spike* phenotype, and the effects of the mutation on cellular organization in fixed and living epidermal cells.

232 A new *cer* loci, *cer25*, having high water loss and reduced cuticle membrane

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Cuticular waxes cover the outermost layer of all aerial plant organs, and likely provide a physical barrier to water loss. Hence these waxes may play an important role in plant drought resistance. Arabidopsis cuticular waxes are a complex mixture of long chain fatty acids, aldehydes, 1o and 2o alcohols, alkanes, ketones, and esters and their biosynthetic pathway is likewise complex. Likely, many enzymes, regulatory and secretory proteins are involved in wax production. Twenty-four *eceriferum* loci were identified and CER1 (ECERIFERUM), CER2, CER3 and CER6 have been cloned and characterized (Jenks and Ashworth, 1999; Fiebig et al., 2000).

We visually screened a T-DNA-mutagenized population of Arabidopsis (generated by Bressan and Hasegawa) and isolated nine *cer* mutants by their distinct glossy surface. Lines TMJ1 and TMJ64 are alleles of *cer2* and *cer4*, respectively, and both lines TMJ88 and TMJ89 are alleles of *cer6*. Allelism and wax compositional studies are currently underway for TMJ90, TMJ92, TMJ93 and TMJ94. Transpiration rates (Jordan *et al.,* 1984; Jenks *et al*., 1994) were determined for 19 of the existing *cer* mutants, revealing that of the new T-DNA insertion mutants, only *cer25*, representing a new loci, showed rapid water loss rate. Further work revealed that *cer25* has a wax composition similar to wildtype except the amount of each constituent is greatly reduced. In addition, *cer25* has a reduction in cuticle membrane thickness. Thus CER25 may block an early step in wax and cutin synthesis, similar to *bm2* in sorghum (Jenks *et al*., 1994). Stomatal frequency and ultrastructure were similar in both wildtype and *cer25*. Southern blot of advanced generation of *cer25* showed that T-DNA is stably inherited in *cer25* and preliminary genetic analysis suggests that *CER25* may be tagged with a T-DNA insert. Studies are underway to clone *CER25*.

233 Requirements for Salicylic Acid and *NIM1/NPR1* in Resistance-gene Signaling

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Disease resistance in higher plants depends in part on highly specific resistance (R) genes that facilitate pathogen detection and initiate signal transduction pathways that activate defenses against the triggering pathogen. In *Arabidopsis thaliana*, this defense system has been shown to depend in part upon salicylic acid (SA) accumulation and activity of a number of proteins, including the *NIM1* (*NON-INDUCIBLE IMMUNITY1*)/*NPR1* (*NON-EXPRESSER OF PR*) gene product. To gain a better understanding of the role of SA and *NIM1*/*NPR1* signaling in a variety of R-gene mediated resistance pathways, we assessed R-gene function in SA-degrading (NahG) and *nim1*/*npr1* mutant backgrounds. We found that all R-genes tested required SA accumulation for full function, regardless of whether they encoded leucine-zipper or Toll-Interleukin-1 receptor (TIR) -like proteins, or whether the R-gene was specific to pseudomonad or oomycete pathogens. *NIM1*/*NPR1* function appears to be required only for the TIR class R-gene *RPP5*, which is active against some *Peronospora parasitica* isolates. Curiously, though *NIM1*/*NPR1* transduces SA signals, most R-genes tested, including *RPP8*, require SA accumulation but not *NIM1*/*NPR1* function, demonstrating the existence of an SA-dependent but *NIM1*/*NPR1*-independent signal transduction pathway capable of producing robust resistance. In addition, SA-dependent, *NIM1*/*NPR1*-independent transcriptional response pathways have been identified in our laboratory using cDNA-AFLP, and results from these experiments will be presented as well.

234 The Arabidopsis genome at the Arabidopsis Information Resource (TAIR) available from http://www.arabidopsis.org

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The release of the Arabidopsis genome sequence by the AGI provides a stepping stone to systematically analyze the biology of this plant. We are developing a database infrastructure and tools to capture, associate, and make accessible the data from a variety of sources. We built the first version of this system and are currently putting a large effort into adding information to our database. Presently we are loading the information about the structure and function of gene models and loci. Curation involves associating gene models to publication records, keywords, aliases, community members, map positions, expression patterns, etc. A gene model is defined as any description of a gene with a source. The source can be an external database (e.g. TIGR, GenBank), literature, in-house computational analysis, or a personal communication by a researcher. Structural annotation of a gene model will include the location on a chromosome, intron-exon boundaries, and other features such as UTRs. Functional annotation of the gene model will include annotations to Gene Ontology terms, with an emphasis on associations made from the current literature. A locus is defined by a physical unit on the sequenced genome corresponding to a transcribed region. Therefore one or more gene models could be associated to a single locus with a chromosome-based name (e.g. AT2G33450). Once the association of gene models to a locus is made, other sequenced elements such as EST(s), polymorphisms including insertions and deletions, mutant alleles, and array elements will also be associated to the locus. Each annotation will be tagged with information about its source. The gene model and locus data will be accessible from a text based search interface as well as from our MapViewer (www.arabidopsis.org/servlets/mapper). We are currently developing a SequenceViewer that will allow researchers to search and browse any sequenced element in a graphical format.

235 *VACUOLELESS1* is an essential gene required for vacuole formation and morphogenesis of the Arabidopsis embryo

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Plant cells are characterized by the presence of a large central vacuole which in differentiated cells accounts for more than 90% of total volume. The role of vacuoles in plant development has not been directly assessed previously, due to the lack of mutants devoid of these compartments. We now show that inactivation of the Arabidopsis gene *VACUOLELESS1* leads to the complete loss of vacuoles. In contrast to yeast mutants that lack a vacuolar compartment and are viable, loss of the plant vacuole leads to embryonic lethality. VCL1 encodes the Arabidopsis ortholog of yeast Vps16p, is peripherally localized to the vacuolar membrane, and interacts directly with the Sec1p homolog AtVPS33. Based on these results, we propose that VCL1 forms part of a protein complex that mediates homotypic and heterotypic fusion at the plant vacuole.

236 Rar1 links Sgt1, an essential component of SCF-Ubiquitin ligase complex.

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The RAR1 gene represents a convergence point in the disease resistance signalling triggered by many resistance genes in barley. Mutations in RAR1 abolish the oxidative burst and cell-death associated with an incompatible interaction with powdery mildew. The predicted amino acid sequence of RAR1 revealed two tandem blocks of 60 amino acids (CHORD I and II) each comprising an invariant arrangement of six cysteines and two histidines. Each CHORD domain can independently bind a zinc ion. Analysis of deduced full length RAR1 sequences from diverse species revealed that the critical amino acids in the two CHORD domains and the physical spacing between them were retained across phyla thus strongly indicating a functional conservation. Yeast two-hybrid data has indicated that CHORD-II interacts with the Arabidopsis SGT1. The highly conserved yeast SGT1 has previously been shown to have an essential role in kinetichore function and the activation of SCF, (Skp/Cull/F-box) ubiquitin-proteasome complexes regulating cell cycle. Immunoprecipitation data in barely has shown that RAR1 and SGT1 do interact in vivo. Similar experiments are being carried out to verify that this interaction is also true in Arabidopsis. The functional role of RAR1/SGT1 complex will be discussed. *Arabidopsisin vivo*

237 Ectopic b-type cyclin expression is sufficient to switch from endoreduplication to mitotic cycles in Arabidopsis trichomes

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Cell differentiation is frequently accompanied by a switch from a mitotic division cycle to an endoreduplication cycle in which DNA replication continues but cell division does not take place. In plants more than 80 percent of all angiosperm species undergo endoreduplication. The underlying mechanism, however, is poorly understood. One attractive scenario is that endoreduplication results from a simple short cut of the mitotic cell cycle. To test this hypothesis we misexpressed two mitotic cyclins in *Arabidopsis* trichomes, a model system for endoreduplicating cells in plants. Here we demonstrate that transgenic expression of CYCLIN B1;2 can drive the endoreduplication cycle into a mitotic cell cycle, transforming the single-celled into multicellular trichomes. This seems to be specific for CYCLIN B1;2 since the expression of CYCLIN B1;1, another member of the b-type cyclins, caused no deviation from the endoreduplication cycle. Mulitcellular trichomes also arise in the *siamese* mutant. Since we could not detect CYCLIN B1;2 mRNA in *siamese* we propose that in addition to the repression of mitotic cyclins other factors are necessary to control the switch from a mitotic to an endoreduplication cycle.

238 A Role for LKP2 in the Circadian Clock of Arabidopsis

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The ZEITLUPE gene was identified in a screen for circadian mutants in Arabidopsis and mutations in this gene were shown to cause increases in period length, short hypocotyls in red light, and late flowering under long days. Characterization of a deletion mutant exhibiting a late flowering phenotype resulted in the identification of a related gene named FKF1. These 2 genes contain a unique combination of proteins motifs, a PAS domain at their N-terminus followed by an F-box motif and 6 kelch repeats at their C-terminus. A third member of the ZTL gene family was identified in the Arabidopsis genome and was named LKP2 for LOV, kelch protein-2. A cDNA was isolated corresponding to this gene and plants over-expressing LKP2 were generated. The over-expression of LKP2 resulted in arrhythmic phenotypes for leaf movement rhythms in continuous light, CAB2::LUC expression in continuous light, and CCR2::LUC expression in both continuous light and continuous dark. Seedlings over-expressing LKP2 also exhibited long hypocotyls under multiple fluences of both red and blue light, and a late flowering phenotype under long day conditions. Results from RT-PCR showed that the LKP2 mRNA is not regulated by the circadian clock and was detected in all tissues examined. These results suggest that LKP2 functions either within or at least very close to the circadian oscillator in Arabidopsis and a model will be presented for its mode of action.

239 Cytokinesis-defective mutants of Arabidopsis

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We have characterized a large collection of cytokinesis-defective mutants of Arabidopsis. At the seedling level, the mutants are characterized by a rough surface layer with bloated cells, as has been described for *keule*[1] and *knolle*[2] mutants. Histological sections reveal cell wall stubs, gapped walls and multinucleate cells. These defects were observed in dividing as opposed to vacuolate cells, which defines these lines as cytokinesis-defective and distinguishes them from mutants with weakened walls which could break during cell expansion. Although all the lines have identical phenotypes at a cellular level, they have differential effects on stomatal and post-embryonic development. For example, while genes such as *KEULE* are required for cytokinesis in all somatic cells, including stomatal guard cells and their precursors, other cytokinesis-defective mutants have normal stomata. Similarly, some lines could be regenerated in tissue culture, whereas others including *keule* and *knolle* could not, suggesting that some genes are required during embryogenesis but might be dispensable thereafter. Cytokinesis-defective lines have a number of additional phenotypes distinct from cytokinesis. By comparing a large number of different lines, we conclude that secondary consequences of a primary defect in cytokinesis include (1) organ fusions,(2)anomalies in organ number,(3)anomalies in cellular differentiation, and (4)perturbations of the nuclear cycle.

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240 Function of the LHY gene within the Arabidopsis circadian clock

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Many aspects of physiology and metabolism show rhythmic variations with 24h periods. These rhythms persist in constant conditions, reflecting regulation by endogenous circadian oscillators. The molecular mechanism of the circadian clock of higher plants is not yet known, but a number of clock-associated genes have been identified in *Arabidopsis*. The *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED-1* (*CCA1*) genes encode rhythmically expressed single MYB transcription factors, which exhibit several properties of circadian clock components. Constitutive overexpression of *LHY* and *CCA1* transcripts caused arrhythmic expression of clock-controlled genes (*CAB* and *CCR2*) and arrhythmic leaf movements under both constant light and darkness. This suggested that *LHY* and *CCA1* rhythmicity was required for the function of the circadian oscillator. Both genes repressed their own transcription, forming a negative feedback loop, and down-regulated each other's expression. These results suggested that *LHY* and *CCA1* may encode redundant components of a negative feedback loop, which may constitute the oscillatory mechanism of the circadian clock.

In support of this hypothesis, mutants lacking either *CCA1* or *LHY* function exhibited similar, short-period phenotypes. So, neither gene was required for the function of the circadian oscillator. To further test the redundancy of these genes, double null mutants (*lhy-11 cca1-1*) have been constructed, and their rhythmic phenotypes were assayed. These plants exhibited arrhythmic leaf movements in constant light. To assay rhythmicity at the molecular level, several clock-regulated luciferase reporter genes were transformed into these plants and assayed in vivo using a photon-counting camera. Rhythmic luminescence expression was detected, however these rhythms had an abnormal phase and dampened within two to three cycles of transfer to constant light conditions. These results imply that *LHY* and *CCA1* may not encode components of the circadian oscillator, although it is possible that genetic redundancy extends beyond these two genes. But they play an important role in sustaining free-running rhythmicity in constant conditions.

241 A cytology-based screen for Arabidopsis mutants with altered callose depostition in response to a non-host fungal 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 in both epidermal and mesophyll cells. While most spores were arrested at penetration, 6% of spores formed haustoria and produced branched hyphae. Haustoria were encased in callose and easily visualized with analine blue staining. In contrast, host resistance occurred late, with callose deposition only at the papillae.

The callose response difference between host and non-host resistance was used as a marker to screen for mutants with an altered callose deposition in response to the non-host mildew. 12,000 EMS-mutagenized plants were screened at 1dpi under UV with analine blue staining, and 500 plants chosen for re-testing. Several aberrant phenotypes were isolated with respect to wild-type: decreased callose deposition, increased widespread callose deposition, increased local callose deposition around penetration events, and plants with a high frequency of fungal penetration events. The last class has been re-tested and seven putative mutants that exhibit an increase in fungal penetration have been identified. These penetration putative mutants will be studied further, and re-testing of plants in the other classes is underway.

242 Positional Cloning and Genetic Analysis of GUN1, a Gene in the Plastid-Nuclear Signal Transduction Pathway of Arabidopsis

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Chloroplast biogenesis requires the coordinate expression of nuclear and chloroplast genes. There is evidence that a chloroplast signal controls the expression of a subset of nuclear genes that function in photosynthesis. The nature of the signal, and the means by which it is relayed to the nucleus, are unknown. Arabidopsis nuclear genes, GUN genes (genomes uncoupled), are required for the coordination of the expression of nuclear and chloroplast genomes. gun mutants inappropriately express photosynthesis-related nuclear genes when chloroplast development is inhibited. The mutants were isolated using a transgenic Arabidopsis line that contains a CAB3 promoter-reporter fusion construct, and have been determined to fall into five complementation groups. Of these, the gun1 mutant exhibits the most uncoupled phenotype and, in addition, has a de-etiolation defect, which results in slower chloroplast development and the slower accumulation of chlorophyll and light-regulated mRNAs. In order to gain a molecular understanding of the chloroplast-to-nucleus signal transduction pathway, we have undertaken the positional cloning of GUN1.The GUN1 gene has been mapped to a 0.3 cM interval on chromosome 2 between nga 361 and COP1. Northern blot analyses have shown that plants with the gun1 mutation have reduced expression of an alternative transcript of a poly(ADP-ribose) polymerase (PARP) gene. Furthermore, treatment of wild-type Arabidopsis plants with the PARP inhibitor 3-aminobenzamide (3-AB) results in a CAB overexpression phenotype. Together, these data suggest that GUN1 is a truncated version of PARP (s-PARP). Complementation testing is underway to confirm this hypothesis.We have also created a number of double mutants between gun1 and other mutants that affect chloroplast development in Arabidopsis. Genetic analysis of the det1gun1 double mutant shows that det1 is epistatic to gun1. These data suggest a model where DET1 is necessary for the activation of a factor that induces CAB expression, and that this inductive factor is modified by PARP during photo-oxidative stress.

243 Expression and Localisation of Calcineurin B-like proteins in Plant Cells

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Until recently the protein phosphatase calcineurin, known to be involved in NaCl tolerance in yeast, remained elusive in plant cells. The identification of a role for calcineurin in plant salt tolerance and the identification of calcineurin B-like proteins (*At*CBLs) in *Arabidopsis* has increased interest in this class of proteins. It is becoming clearer that they have a role interacting with a family of protein kinases, however their sub-cellular localisation remains uncertain.

Eight known CBLs have been identified and four contain the known myristic acid binding motif (MGXXXSK). Additionally, one contains a putative ER signal peptide. Microscopical and biochemical techniques are being used to determine how these motifs affect sub-cellular localisation and function.

Overexpression of *At*CBL1:GFP constructs in BY-2 cells indicate that this protein is localised at the plasma membrane and further experiments expressing fusion proteins with mutated or truncated myristic acid binding sites and other members of the *At*CBL family are underway. These GFP fusions will also allow us to observe changes in localisation in response to various stresses. Transient expression experiments have suggested that the myristic acid site when fused to the N-terminus of the neutral carrier protein: phosphinothricin acetyl transferase (PAT) is not sufficient for membrane targeting, indicating other targeting signals may be required.

Using the pMAL system, *At*CBL1 has been successful expressed and purified from E.coli, allowing further biochemical characterisation to be carried out and a screen for interacting proteins to been initiated.

244 Mutational analysis of RPM1 function.

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Plants, like any organism, are in constant combat in the natural environment. To deal with this threat they have evolved ways to recognize pathogens and provide a programmed response. In our group, we work with *RPM1*, a resistance gene from *Arabidopsis thaliana* that recognizes -direct or indirectly- AvrRpm1 or AvrB proteins from *Pseudomonas syringae*. Their interaction initiates a signaling cascade that culminates in resistance of the plant to the bacterium. To study this interaction, we set up an inducible system that allows us to express avrRpm1 inside the plant cell. With this system, we screened and found mutants that have loss of recognition to avrRpm1 (lra). There are 5 complementation groups defined with this screening. The most frequent gene found was RPM1, with approximately a hundred alleles. The clustering of this mutations, and the significance of the numbers found in the screening will be discussed.

245 Fine scale mapping of quantitative trait loci in *Arabidopsis thaliana*

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Many important traits of plants show quantitative variation between individuals. These traits are considered to be under the influence of many genes and also the environment. The environmental variation makes reliable association between a genotype and a specific phenotype very difficult. Consequently it is very difficult to genetically map these quantitative trait loci (QTL) with great accuracy. Typical mapping attempts use dense molecular marker maps and large mapping populations. These studies have generally resulted in map positions with confidence intervals that span 20-30 cM. A much finer scale of mapping would allow a map based cloning approach to study the genes that affect quantitative traits. *Arabidopsis thaliana* is a model flowering plant and closely related to important crops in the Brassicacea family. Recombinant inbred lines from the cross between the ecotypes Columbia-5 and Niederzenz-1 have been used to map QTL influencing growth and flowering time. The work presented includes the results of this QTL analysis and the current achievements in developing overlapping substitution lines for fine scale mapping of QTL.

246 Hormonal interactions in the regulation of cell death

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Ozone (O_3) has been established as a convenient tool in probing the role of the oxidative burst, reactive oxygen species (ROS), and also the general mechanisms of the hypersensitive cell death. An ozone, superoxide, and virulent pathogen-sensitive Arabidopsis mutant, radical-induced cell death, *rcd1* (Plant Cell 12:1849), was utilized in elucidating the interactions of ethylene (ET), jasmonate (JA) and salicylate (SA) signaling with ROS in the regulation of cell death. ET enhanced cell death in both *rcd1* and Col-0, and functional ET signaling, demonstrated with *ein2* and *rcd1/ein2*, was required for spreading of the cell death. In an opposite manner, JA reduced the spreading cell death in *rcd1*. Similar results were obtained with other mutants. ET-insensitive *ein2*, SA-insensitive *npr1* and SA-degrading NahG were tolerant, and ET overproducing *eto1* and JA- insensitive *jar1* and *coi1* were sensitive to O_3. Double mutants were created to further elucidate interactions of these hormones. *rcd1/ein2*, *rcd1*/nahG, *rcd1/jar1*, *ein2/jar1*, nahG/*jar1* and *ein2*/nahG revealed a specific role of each of the signaling pathways, and epistatic relationships in the O_3-induced cell death. The results suggest that interactions between ET, SA, and JA signaling regulate spreading of cell death, and that RCD1 function is involved in the interaction of these pathways. Furthermore, results indicated that JA signaling is involved in regulating the degree of plant ethylene sensitivity affecting cell death, gene expression, and also plant growth and development in general. A model for the contribution of ET, SA and JA in the regulation of cell death is presented.

247 Isolation and characterisation of Mg transport genes from *Arabidopsis thaliana*

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Magnesium (Mg) is the most abundant divalent cation in biological systems and plays a critical role in many biological processes. I have isolated a novel *Arabidopsis* Mg transporter *(AtMGT10)* by complementation of a mutant yeast strain (CM66) in which the Mg transport system (ALR) has been deleted. *AtGMGT10* was identified as an effective suppressor of the alr mutant phenotype. Atomic absorption spectroscopy measurements shows that the expression of *AtMGT10* increases Mg uptake of the yeast mutant strain CM66. The presence of the AtGMGT10 protein in the plasma membrane was confirmed by Western blots. *AtMGT10* encodes a protein that contains structural features similar to bacterial (CorA) and yeast (ALR1 and ALR2) Mg transporter genes. It is most similar to the yeast MRS2 gene, recently identified as encoding a magnesium uptake system in yeast mitochondria. Comparison of the *AtMGT10* sequence with the DNA sequence database has identified several homologous ESTs derived from human, mouse and *Arabidopsis thaliana*. Overexpression of *AtMGT10* in yeast alters sensitivity to metal ions, suggesting it transports a range of cations. The transport characteristics of *AtMGT10* have been investigated by expression in *Xenopus* oocytes. Preliminary results of two-electrode voltage-clamp studies have identified an inward current at 1 mM Mg. I am currently undertaking further functional analysis of *AtMGT10* in the oocyte expression system and in *Arabidopsis thaliana*.

248 *TITAN* Gene Functions During Seed Development

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The *titan* mutants of *Arabidopsis* exhibit striking defects in seed development. The defining feature is the presence of abnormal endosperm with giant polyploid nuclei. Two gene products are known: TTN5 is related to the ARF (ARL2) class of GTP binding proteins; TTN3 is a member of the SMC2 family of chromosome scaffold proteins (condensins). Here we describe 15 *titan* mutants recovered from a forward screen of T-DNA insertion lines. Six genes were cloned using TAIL-PCR, including four disrupted twice in the population. Two genes encode chromosome scaffold proteins that underscore the importance of chromosome integrity during endosperm development. Another gene encodes a protein that may interact with TTN5 during seed development and should provide insights into ARL function in plants. Additional *TTN* gene products appear to be involved in protein degradation pathways and plant senescence. Together these results suggest that *TTN* genes act in overlapping pathways that influence chromosome mechanics and cytoskeletal organization during seed development.

249 Characterisation of *LEP* and *VAS*: two closely linked genes affected by activation tagging in the *lettuce* mutant

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The T-DNA tagged *lettuce* mutant exhibits the formation of leaves without petiole and an increased vascular bundle size in all aerial organs. The T-DNA insert linked with these dominant phenotypes caused an altered expression of two closely linked genes, *LEP* and *VAS*, arranged in a tandem orientation. The leafy petiole phenotype is caused by activation tagging of the AP2/EREBP like transcription factor *LEP* and consists of the conversion of the leaf petiole region into leaf blade (Development 127 (2000): 4971-4980). In wild-type plants, *LEP* is expressed specifically in leaf primordia and young developing leaf blades. *LEP* expression pattern was unaltered in *lettuce* indicating that the activation tagging caused tissue specific increased *LEP* expression. Interestingly, in wild type *LEP* was expressed throughout the complete leaf primordium even at the stage where morphologically the petiole could be distinguished from the leaf blade. Therefore, LEP activity must be regulated on protein level, either by interaction with other transcription factors specific for the leaf blade region or by interaction with a negative regulator specific for the petiole region. Yeast two-hybrid analysis has been initiated to screen for such interacting partners of LEP. A revertant screen has been performed to isolate target genes of LEP. Out of 50,000 M2 EMS mutagenised seeds, eight lines have been selected exhibiting a wild-type leaf development in the presence of the activation tagged *LEP* gene and analysis of these revertants is in progress.

The vascular phenotype is caused by activation tagging of *VAS* and consists of an increased cell number for all cell types comprising the vascular bundles. In wild-type plants *VAS* expression can be detected in the vascular bundle of roots and the lower part of hypocotyls using promoter-GUS fusions. RT-PCR analysis showed that *VAS* is expressed in all plant organs. Activation tagging of *VAS* caused increased *VAS* expression in the vascular bundles of all aerial organs.

In order to study the exact role of *LEP* and *VAS* in plant development, we generated repression transgenics and are currently screening for insertional mutants to obtain loss-of-function lines.

250 Characterization of the pathosystem involving *Arabidopsis thaliana* and *Verticillium dahliae* Kleb.

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Verticillium dahliae Kleb. is a soil-borne fungal pathogen causing vascular disease and severe yield and quality losses in a broad range of fruit and vegetable crops. The fungus penetrates the host through the roots and spreads systematically through the xylem. It generally lacks host specificity and no significant differences in the systemic colonization are found in resistant and susceptible cultivars. Using an isolate of the fungus that is pathogenic on crucifers, we surveyed several *Arabidopsis* accessions for susceptibility. No immunity was found and the disease symptoms included leaf chlorosis and stunting and in some ecotypes the formation of multiple stems. Using an *in vitro* inoculation system, we characterized more susceptible ecotypes as having stronger anthocyanin accumulation, more severe stunting and early induction of flowering and senescence. Analysis of crosses indicated tolerance can be conveyed by a single dominant gene. The screening of an activaction tagged T-DNA population of the tolerant C-24 ecotype generated three mutants characterized by early dying in response to the fungus. All the mutants presented further unique characteristics indicating separate affected genes, one being also early flowering, another dwarf and sterile. Molecular and genetic characterization of the mutants will be presented.

251 The Pursuit Of Genes Involved In Xyloglucan Biosynthesis

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There has been increasing interest in the role of the cell wall during plant development and in the wall's dynamic nature. The identification of enzymes that synthesize cell wall carbohydrates is necessary to address the regulation and function of wall components and the mechanisms of wall assembly. We have focused our efforts on identifying enzymes involved in the synthesis of xyloglucan, the most abundant hemicellulose in plants. The glycosyltransferase that adds the terminal fucose to xyloglucan was identified based on a biochemical assay. However, there are no available assays for the remaining enzymes expected to be involved in the synthesis of this glycopolymer. Instead, we took a bioinformatics approach to identify potential candidate genes and will use reverse genetics to deduce their function. Candidate genes are expected to be highly expressed or present in cells that are undergoing rapid expansion and to be absent from cells that are producing secondary cell walls. Both developing cotton fibers and differentiating zinnia tracheids undergo a stage of rapid cell expansion that is temporally distinct from when these cells produce secondary cell walls. Screening mRNA populations from these cells at different developmental times has allowed us to identify genes that meet our criteria. One candidate gene from developing cotton fibers is predicted to be a Golgi type II membrane protein and a member of glycosyltransferase family 8 (http://afmb.cnrs-mrs.fr/~pedro/CAZY/db.html). The Arabidopsis homologue of this gene has been identified, and we are pursuing this candidate's function by RNA inhibition and gene knockouts.

252 SIAMESE, a regulator of the endoreduplication cell cycle, results in multicellular trichomes.

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Endoreduplication is a variant of the cell cycle that occurs in a wide variety of organisms. During endoreduplication cycles (endo cycles), DNA is replicated without cellular or nuclear division. One Arabidopsis cell type exhibiting endoreduplication is the trichome (leaf hair). Recessive *siamese* (*sim*) mutations result in multicellular trichomes in place of the unicellular trichomes produced by wild-type plants. Individual nuclei of a multicellular trichome have a reduced level of endoreduplication relative to wild-type, indicating that wild-type *SIM* may function to suppress mitosis during the switch to the endocycle early in trichoem development. *SIM* is also required for light-regulated endoreduplication in the hypocotyl, although it is not required for all endoreduplication events. Putative genetic modifiers of *sim* have been isolated, including both phenotypic suppressors and enhancers. These new mutants are currenttly being characterized. Progress toward isolating the *SIM* gene will be reported.Supported by NSF award IBN-9728047.

253 An Analysis of *Tangled1,* a Gene Required for the Spatial Control of Cytokinesis in Maize

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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 adematous 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. Recently we have identified a gene in *Arabidopsis thaliana,* which encodes a protein that is highly related to TAN1 and have identified a plant containing a T-DNA insertion in this gene. Work on the characterization of this gene will be presented.

254 *tfa "things fall apart,"* a Novel Mutant Regulating Cell Separation

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We have identified several ectopic cell separation mutants that we have designated *tfa "things fall apart*." These mutants are all characterized by unregulated cell separation in epidermal, cortical and vascular tissues. This process is most severe in young seedlings affecting the hypocotyl, cotyledon, and first true leaves. Crosses among the mutants indicate that these genes are alleles, and we have designated them *tfa1-1, tfa1-2* and *tfa1-3*. *tfa1-1* and *tfa1-3* were identified in T-DNA insertion populations, and *tfa 1-2* was identified from an EMS screen. We were able to clone *TFA1* by probing a C-DNA library of *tfa1-3* with T-DNA. Sequencing identified a hypothetical protein approximately 850AA, and indicated T-DNA insertions in the third intron (*tfa1-1*) and the forth exon (*tfa1-3*). Molecular complementation of *tfa1-1* with *TFA1* restores wild type phenotype confirming identification of the gene. *TFA1* is predicted to be highly hydrophilic and form coil-coil structures. A knockout in a homologous gene that we have designated *tfa2-1* has been identified and the analysis of interactions with *tfa1* is in progress. We propose that there are several regulatory pathways affecting cell separation programs in the plant. *TFA1* may represent a gene regulating global repressor systems, while *TFA2* may regulate a more tissue specific program.This work was funded by USDA grants 9835301-6764 and 0035301-9085.Corresponding author: spatters@facstaff.wisc.edu

255 Katanin, microtubules and cell specification in the *Arabidopsis* root

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The *Arabidopsis* primary root has a simple radial organisation consisting of different cell types in concentric rings surrounding the stele tissue. This radial pattern is set down during embryogenesis and thought to be maintained by positional cues. The epidermis is sub-specified into two cell types, hair forming cells and non-hair forming cells. These cell types differ in their location relative to the underlying cortical cell walls, indicating a role for cell wall localised positional information. The nature of these positional mechanisms is unknown.

The *ectopic root hair 3* mutant (*erh3*) has defective epidermal sub-specification; ectopic root hairs form in non-hair cell positions, ectopic non hairs form in hair cell positions. The expression of non-hair cell specific molecular markers is altered in the meristem indicating an early role for the *ERH3* gene. Additionally the defective expression pattern of cortical/endodermal and lateral root-cap specific markers in *erh3* roots suggests disrupted radial patterning.

Positional cloning of the mutated gene identified a single copy gene encoding the p60 subunit of a katanin-like protein. In animal systems, katanin functions as a microtubule-severing protein and is supposed to be involved in mitosis and microtubule dynamics.

We examined microtubule organisation in the meristem and the differentiated region of the epidermis in wild type and *erh3-2* mutant roots to investigate the role of katanin in plants. Our results indicate that microtubule orientation is defective in *erh3* mutant suggesting that a microtubule dependent signalling process is required for correct cell specification. Further characterisation of this mutant will provide an insight into this signalling process.

256 MOR1: a novel structural microtubule associated protein expressed throughout development.

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We identified the *MICROTUBULE ORGANIZATION 1* (*MOR1*) gene by mapping, complementation and sequence analysis of two temperature-sensitive mutant alleles. *MOR1* is a large gene of ~14kb, comprising at least 53 exons with a coding mRNA of ~6kb. We have isolated no constitutive *mor1* mutants and suspect that constitutive loss of function would be lethal. Southern blot and genome sequence analysis confirmed that *MOR1* is a single copy gene, although two pseudogenes lacking N and C termini were identified. We speculate that the size and complexity of the *MOR1* gene, together with the lack of redundancy, might allow several functions to occur through regulation at the transcriptional level, for example alternative splicing. Phenotype analysis of the mutants suggests that *MOR1* is expressed throughout development and in virtually all organs. RT-PCR analysis confirms *MOR1* expression in roots, shoots, rosettes, stems, siliques and flowers. The rapid disruption of cortical microtubules in the mutants at the restrictive temperature suggests the structural integrity of MOR1 protein rather than a transcription event is affected. RT-PCR analysis showed that *MOR1* is expressed in wild-type and mutant tissues at both the permissive and restrictive temperatures. The deduced MOR1 protein sequence revealed several intriguing features including at least 10 HEAT repeats, two nuclear localization signals, and a consensus microtubule binding motif. Further dissection of this structural MAP with information from the mutants will help us understand the specialized features and functions of microtubule arrays in plant cells.

257 Diversity of *Arabidopsis* Genes Encoding Precursors for Phytosulfokine-α, a Peptide Growth Factor

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Phytosulfokine-alpha (PSK-alpha), a peptide growth factor, was originally isolated from conditioned medium of asparagus mesophyll cell cultures. PSK-alpha promotes plant cell proliferation, enhances chlorophyll synthesis and growth of seedlings, induces formation of adventitious roots and buds, stimulates tracheary element differentiation, and reinforces the frequency of somatic embryogenesis. Four genes, *AtPSK1*, *AtPSK2*, *AtPSK3* and *AtPSK5*, encoding precursors of PSK-alpha have been identified in *Arabidopsis*, suggesting that the *Arabidopsis* genome possesses a dynamic gene family for PSK-alpha precursors. The predicted precursors have N-terminal signal peptides, with only a single PSK-alpha sequence, close to their carboxyl termini. All precursors exhibit dibasic processing sites flanking PSK-alpha, analogous to animal and yeast prohormones. Although the PSK domain including the sequence of PSK-alpha and three amino acids preceding it are perfectly conserved, the precursors bear very limited similarity, suggesting a new level of diversity among polypeptides that are processed into same signaling molecule in plants, a scenario not found in animals and yeast. Transgenic *Arabidopsis* cells expressing a mutant of either *AtPSK2* or *AtPSK3* cDNAs secreted unnatural [Ser ^{4}]PSK-beta. Both *AtPSK2* and *AtPSK3* were demonstrably expressed not only in cultured cells but also in intact plants, suggesting that PSK-alpha may be essential for plant cell proliferation in vivo as well as in vitro, and that these two genes may redundantly act. Such pairs of genes allow functional stabilization in case one becomes switched off. The evolution of *Arabidopsis* apparently involved a whole-genome duplication, followed by subsequent gene loss and extensive local gene duplications, giving rise to a dynamic genome. Interestingly, the *AtPSK2* and *AtPSK3* genes are located in the large duplicated segments of chromosomes II and III, suggesting a derivation from the same ancestor but divergence after the duplication event. The long period of time over which genome stabilization has occurred has, however, provided ample opportunity for divergence of functions of genes during through duplication.

258 The cis-acting signal for degradation of Aux/IAA proteins

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The Aux/IAA proteins are a family of short-lived proteins whose mRNAs rapidly increase in the presence of exogenous auxin. We previously showed directly using a radioactive pulse-chase analysis that an Aux/IAA protein, PSIAA6, in fusion with firefly luciferase (LUC) targeted the protein for rapid degradation (Worley et al, 2000). Using Aux/IAA luciferase LUC fusion proteins, we determined amino acid sequences required for this rapid degradation in both transient expression assays using tobacco protoplasts and in cycloheximide chase experiments using transgenic Arabidopsis. A sequence of 13 amino acids based on conserved sequences found in Domain II of all Aux/IAA proteins was found to be sufficient in transient assays for low protein accumulation. Mutagenesis of this region and transient expression of mutant 13aa::LUC fusion proteins defined the necessary amino acids within these 13 amino acids. Substitutions previously found in semi-dominant auxin signaling mutants were made and tested in the context of the 13 amino acid LUC fusion protein. All had dramatic effects on protein accumulation. The effects of single alanine substitutions at almost all positions were tested. In some cases where single alanine substitutions had no effect, multiple alanine substitutions affected protein accumulation. The wild-type 13aa:LUC fusion was expressed in transgenic Arabidopsis. These amino acids were not sufficient for the equivalent rapid degradation seen for a full length PSIAA6:LUC protein in transgenic plants, although 13aa::LUC was degraded significantly faster than LUC alone. Addition of 56 amino acids of PSIAA6 surrounding these13 amino acids increased the degradation rate to within approximately 2-fold that of the full length protein. In contrast, addition of the N-terminal 73 amino acids of PSIAA6 that includes the 13 amino acid sequence produced a LUC fusion protein that was degraded as fast as the full length fusion protein

259 T-DNA insertion in a putative helicase promoter causes late-flowering in *Arabidopsis thaliana* (L.).

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The switch to flowering involves a major change in the pattern of differentiation at the shoot apical meristem leading to the development of the floral organs. Analysis of the responses of different mutants to the environment together with the studies of their genetic interactions have resulted in a multiple pathway model showing the complex integration of endogenous signals and environmental conditions during the floral transition.

In a screen for late-flowering under long-day conditions, we isolated a T-DNA tagged *Arabidopsis* mutant line with a particular phenotype: a part of the population presented an intermediate phenotype, flowering between 8 and 15 days after the wild-type while a smaller proportion gave sterile flowers 15 days later. The T-DNA insertion was found to be located in the promoter region of a putative helicase, which we named *Heli1*. The universal presence of helicases in the living kingdom reflects their fundamental importance in DNA and RNA metabolic processes, including replication, recombination, DNA repair, transcription, translation and RNA splicing. So given the diverse functions of those proteins, it is difficult to predict a specific function for heli1. Further genetic analysis revealed that the sterile plants where homozygote for the T-DNA and that the intermediate phenotype was related to the hemizygotes, indicating that a reduction of the heli1 activity seems to be sufficient to delay flowering time. This requires that heli1 activity, for example its transcription, is tightly regulated, and predicts that differences in flowering time are caused by changes in the levels of *Heli1* mRNA. A similar observation has been done in *Arabidopsis* where the disruption of an RNA helicase/RNA III gene caused unregulated cell division in floral meristems (Jacobsen S.E., Running M.P. and Meyerowitz E.M., 1999, Development 126 : 5231-5243). The next step of our study shall be to characterise an tissue-specific or transient expression of the putative helicase and then the identification of the upstream and the downstream targets of this gene's product which might help to establish its regulatory role during floral transition or development.

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260 Identification of genetic interactions with the transcriptional activator ABI3

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Loss-of-function mutations in the B3-type transcriptional activator *ABI3* of Arabidopsis result in seed phenotypes ranging from incomplete late embryogenesis to increased insensitivity to ABA at the level of seed germination (Nambara et al., 1995). Although this genetic data indicates *ABI3* is an important regulator of seed development, recent expression studies suggest this gene may have functions outside of the seed (Rohde et al., 1999). To further understand *ABI3* function we have devised a genetic screen to identify genes that regulate *ABI3* expression outside of seed development. The premise of the screen is based on two observations; first, loss-of-function mutations in the era1 gene, which enhance the ABA response of Arabidopsis, are phenotypically suppressed in *abi3* mutants and second, *era1* plants show an enhanced expression of *ABI3* in lateral root primordia. Using the genetic relationship between *ERA1* and *ABI3* and the *ABI3* expression profile as a marker we screened for supressor mutations of the era1 germination phenotype in an *era1 ABI3::GUS* line. In the M3 generation, mutants were rescreened for aberrant expression of *ABI3* in the roots and lateral root defects. Using these criteria, we have identified a variety of mutations that affect; *ABI3::GUS* expression in the roots, mutations that affect root patterning and development, mutations in root hair production and mutations that arrest seedling development. These mutations are now being classified into groups based on morphological root phenotypes and *ABI3* expression patterns.

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261 Forward genetic studies of glutamate receptor genes in Arabidopsis

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In the animal brain, ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that transmit synaptic signals necessary for a variety of functions including vision and memory. Genes with high sequence similarity to animal iGluRs have been identified in Arabidopsis. We have taken a pharmacological approach to uncover the role of Arabidopsis glutamate receptor (AtGLR) genes, by examining the effects of BMAA [S(+)-beta-Methyl-alpha, beta-diaminopropionic acid], a cycad-derived iGluR agonist, on Arabidopsis morphogenesis. When grown in the presence of BMAA, Arabidopsis seedlings show a two to three fold increase in hypocotyl length and a significant inhibition of cotyledon opening. The effect of BMAA on hypocotyl elongation is light-specific and can be reversed by the simultaneous application of glutamate, the native iGluR agonist in animals. A genetic screen was devised to isolate Arabidopsis mutants with a BMAA insensitive morphology (bim). When grown in the light on BMAA, bim mutants have shorter hypocotyls then wildtype. When grown in the dark in the absence of BMAA bim mutants could be grouped into three classes based on their morphology. Class I bim mutants have a normal, etiolated morphology, similar to wildtype plants. Class II bim mutants have shorter hypocotyls and closed cotyledons when grown in the dark. Class III bim mutants have short hypocotyls and open cotyledons when grown in the dark, resembling the constitutively photomorphogenic mutants (cop, det, fus, shy). Further analysis of the bim mutants should help define whether plant-derived iGluR agonists target glutamate receptor signaling pathways in plants.

262 Interactions among loci regulating ABA response and seed development

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Genetic and physiological studies have shown that the Arabidopsis abscisic acid (ABA)-insensitive *ABI3, ABI4, ABI5* and *LEC1* and *FUSCA3* loci regulate many aspects of embryonic and seed development. *ABI3* regulates sensitivity to ABA inhibition of germination, expression of some seed-specific genes, acquisition of dormancy, and desiccation tolerance. The other loci regulate subsets of these responses: all four loci control some embryonic gene expression, but only *ABI4* and *ABI5* affect ABA sensitivity of germination, while *FUS3* and *LEC1* are required for desiccation tolerance. *ABI3, FUS3* and *LEC1* have been shown to interact genetically such that double mutant seeds are highly pigmented and viviparous.Double mutants combining either *abi4* or *abi5* mutations with either *lec1* or *fus3* mutations also show synergistic interactions in enhancing both anthocyanin and chlorophyll accumulation in double mutant embryos, but only the *fus3* and *abi* mutations have synergistic effects on ABA sensitivity of germination. Expression of late embryogenesis abundant (*lea*) genes in dry and immature seeds of these double mutants is in progress.The yeast two-hybrid assay has been used to determine whether any of these genetic interactions reflect direct physical interactions. By this criterion, only ABI3 and ABI5 physically interact with each other, and ABI5 can form homodimers.

263 Functional and Expression Analysis of the Vernalization-Responsive Gene *EARLI1*

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The transition of a flowering plant from the vegetative to the reproductive growth phase is a highly regulated developmental event. Vernalization, the sustained exposure of plants to cold temperature, results in the induction of flowering. A subtractive hybridization approach was used to isolate vernalization-responsive genes from a late flowering ecotype of *Arabidopsis thaliana* based on the premise that transcript levels of such genes would increase with cold treatment and remain high even after removal of the vernalization stimulus. This gene expression screen identified *EARLI1*, as a novel vernalization-responsive gene. To understand the mechanism of vernalization responsive gene activation and silencing, the function and regulation of *EARLI1* are being analyzed. *EARLI1's* regulation is being reconstituted using promoter::GUS fusions in transgenic *Arabidopsis* plants to i) determine the minimal size of the *EARLI1* gene that responds to vernalization, ii) identify the vernalization response element, and iii) identify other regulatory elements. Loss of function *EARLI1* and gain of function *EARLI* transgenic plants are being created to determine how the absence or abundance of EARLI affects flowering time. An analysis of the spatial and temporal patterns of GUS expression regulated by the 5' region of the *EARLI1* gene will be presented, in addition to the current *EARLI1* functional data.

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

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A typical calcium-dependent protein kinase (CDPK) consists of a serine/ threonine protein kinase domain fused to a series of calcium-binding motifs. To date, CDPKs are only found in plants, algae and protists, and have been most extensively studied in plant systems. The *Arabidopsis thaliana* genome sequencing project suggests that the CDPK gene family consists of 34 different members, and represents one of the largest groups of serine/ threonine protein kinases. Members of the family share extensive sequence homology, ranging from ~35% to 95% amino acid identities. Biochemical studies on selected CDPKs confirm that calcium directly regulates their kinase activities (1). Therefore, CDPKs can serve as important sensors and effectors of calcium signals in various physiological processes. There is indeed evidence for involvement of CDPKs in important processes as the synthesis of sucrose, assimilation of nitrate (1), and control of stomatal aperture (2).

In order to better understand in vivo functions of the CDPK gene family, we have applied a reverse genetic approach to a plant model system, *A. thaliana.* In particular, we have been systematically searching for CDPK 'knockout' plants in large transferred DNA transformed populations (3). We present our current data on the isolation of CDPK mutants, and also discuss our progress in detecting and analyzing phenotypic differences between mutant and wild-type plants. Furthermore, to address the issue of functional redundancy among members of the CDPK gene family, we have begun performing genetic crosses between different CDPK mutants, thereby creating plants that are defective in specific combinations of CDPKs. Our studies on CDPK mutant plants are beginning to reveal the contribution of individual and groups of CDPKs to various physiological processes, and will better our understanding of in *planta* functions of this family of protein kinases and their roles in calcium signaling..

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265 The Ethylene Receptor ETR1 Localizes to the Endoplasmic Reticulum of Arabidopsis

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The ethylene receptor ETR1 is composed of ethylene-binding, histidine kinase, and receiver domains. The hydrophobic ethylene-binding domain is also responsible for membrane localization of the receptor. Sequence analysis does not provide information as to which membrane system of Arabidopsis the receptor is localized. We have employed several independent approaches to determine membrane localization of ETR1. Membrane fractionation by two-phase partitioning and sucrose density-gradient centrifugation indicate that ETR1 is associated with the endoplasmic reticulum (ER). To confirm ER localization of ETR1, we affinity-purified a rabbit antiserum against ETR1 to monospecificity and used it as a probe in immunogold electron microscopy. Gold label indicating ETR1 was found in the ER but not in the Golgi apparatus or plasma membrane. Localization was not affected in plants treated with the ethylene precursor ACC or the protein biosynthesis inhibitor cycloheximide, supporting a stable localization of the receptor to the ER. Expression of a truncated receptor in Arabidopsis indicated that the aminoterminal half of the protein was sufficient for ER localization. Our data establish the ER as a novel alternative location for hormone or growth factor receptors, contrasting with the typical localization of receptors to the plasma membrane or nucleus.

266 Brassinosteroid signaling casts a new player DWARF12

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Compared to the flurry of brassinosteroid (BR) biosynthetic mutants, a dearth of mutants defective in the BR signaling pathway has made it difficult to obtain additional components of BR signaling. To find other players in the pathway featuring the receptor kinase BRI1, we have performed extensive screening of EMS-mutant populations and have isolated novel BR-insensitive mutants in *dwarf12*. *dwf12* mutants share many characteristic phenotypes of previously reported BR mutants, such as short stature, short round leaves, infertility, and abnormal de-etiolation. *dwf12* mutants also have a unique phenotype, severe downward curling of leaves. Interestingly, plants heterozygous for these mutations show a dwarf phenotype whose height is intermediate between wild type and homozygous mutant plants, suggesting that the *dwf12* mutations are dominant-negative, gain of function, or haploinsufficent. Further morphometric analysis of the two *dwf12* alleles, *dwf12-1D* and *dwf12-2D,* indicated that the *dwf12-2D* mutation resulted in more severe phenotypes than those of *dwf12-1D*. Similar to *bri1*/*dwf2, dwf12* plants accumulated a significant amount of BRs including brassinolide. We identified DWF12 using map-based cloning. DWF12 encodes a conserved protein predicted to reside in the cytoplasm. We hypothesize that DWF12 acts downstream of BRI in a phosphorylation cascade, ultimately leading to the activation of BR-dependent transcriptional events.

267 Role of phosphorylation of CCA1 by the protein kinase CK2 in the circadian clock of *Arabidopsis thaliana*

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CCA1 is a Myb-related transcription factor that has been shown to be involved in the phytochrome regulation of *Lhcb1*3* gene expression and in the function of the circadian oscillator of *Arabidopsis thaliana* [1, 2]. CCA1 was shown to interact *in vitro* with both α -subunits and β-subunits of the protein kinase CK2, and the reconstituted CK2 enzyme can phosphorylate CCA1 protein *in vitro*[3]. Furthermore, whole-cell extracts from Arabidopsis plants contain a CK2-like activity that can phosphorylate CCA1 [3]. Finally, over-expression of a β-subunit of CK2 shortened periods of circadian rhythms [4]. These results demonstrate that CK2 affects circadian rhythms *in vivo*, and they suggest that the mechanism of this effect is likely to involve phosphorylation of CCA1. We are now in the process of using a site-specific mutational strategy to test whether phosphorylation of CCA1 by CK2 is a mechanism that is normally used as a regulatory element in the circadian clock of Arabidopsis. We used mass spectrometry of CCA1 phosphorylated by CK2 *in vitro* to identify Ser-5 and Ser-6 of CCA1 as phosphorylation targets of CK2. Sequence analysis of the CCA1 protein revealed four additional Sers in conserved CK2 motifs (S/T X X D/E) that could be targets for CK2 phosphorylation. We have mutated all six of these Ser residues, changing them to Ala residues. We then tested whether this mutant protein (mCCA1) could be phosphorylated by CK2 *in vitro*, and we found that it is a poor substrate for CK2-phosphorylation as expected.We have now transformed a line of plants that is null for CCA1 [5] with the mutant form (mCCA1) of CCA1 under the control of a constitutive promoter. If the phosphorylation of CCA1 is important for its function in circadian rhythms, we expect that the phenotype of plants overexpressing the mutant form of CCA1 (mCCA1) that cannot be phosphorylated by CK2 will be different from the phenotype of plants overexpressing the WT form [2]. The results of such experiments will be presented.

1. Wang *et al*. (1997) *Plant Cell* 9:491-507.2. Wang and Tobin (1998) *Cell* 96:1207-173. Sugano *et al*. (1998) *PNAS* 95:11020-254. Sugano *et al*. (1999) *PNAS* 96:12362-66.5. Green and Tobin (1999) *PNAS* 96: 4176-79.

268 AXR1 homologue AXL1 is involved in Auxin response in Arabidopsis.

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The plant hormone auxin regulates many aspects of growth and development. Recent studies have shown that auxin response is mediated through ubiquitin proteosome pathway involving SCF^{TIR1} complex. The activity of SCF complex is known to be modulated by the RUB(ubiquitin-Related protein) modification of cullin, a key component of this protein complex. RUB modification of cullin is initiated by RUB activating enzyme (E1) that consists of an AXR1 and ECR1 heterodimer. AXL1 is a homologue of AXR1 with 80% amino acid identity. Therefore, we hypothesized that AXL1 and AXR1 may be functionally redundant. To test this hypothesis, AXL1 cDNA was cloned and overexpressed in axr1-3 background using CaMV 35S promoter. Overexpression of AXL1 in axr1-3 plants resulted in complementation of the auxin resistant phenotype of axr1-3. Further, AXL1 overexpressed lines had higher level of modified cullin compared to axr1-3 plants. Moreover, we isolated T-DNA tagged knockout mutant line of AXL1. While mutants homozygous for AXL1 (axl1/axl1) did not show any noticeable phenotype, axl1/+ or axl1/axl1 in axr1-12 background showed severe dwarf shoot phenotype that is much more extreme than axr1-12. Taken together these results suggest that AXL1 functions as a subunit of the E1 enzyme involved in the RUB modification pathway.

269 THE UBIQUITIN-RELATED PROTEIN RUB1 IS REQUIRED FOR AUXIN RESPONSE IN ARABIDOPSIS.

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The plant hormone auxin regulates many aspects of plant growth and development. Genetic and biochemical studies have shown that cellular responses to auxin depend upon the function of a ubiquitin protein ligase (E3) called SCF ^{TIR1}. In addition, our studies indicate that the ubiquitin-related protein RUB1 is required for auxin response. Since the only known targets for RUB modification are members of the cullin family, a subunit of SCF-type E3s, we hypothesized that RUB modification of the cullin component of SCF ^{TIR1} may be required for its function. To test this possibility we have performed genetic studies of the RUB-conjugation pathway. Our results indicate that mutations in the genes that encode the two subunits of the RUB-activating enzyme (AXR1 and ECR1) and the RUB conjugating enzyme (RCE1) all result in defects in auxin response. In addition, we find that these mutations affect RUB conjugation to the cullin AtCUL1, suggesting that RUB modification is required for normal SCF ^{TIR1} function.

270 Dissection of ABA signaling pathways in Arabidopsis by global examination of ABA-regulated genes in abi1 and abi1 enhancer abe1 mutants

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The plant hormone abscisic acid (ABA) is important in numerous functions ranging from the establishment of seed development and dormancy to protecting adult plants from a variety of environmental stresses. Several mutants with reduced sensitivity to exogenously applied ABA (*abi*) have been described. One ABA insensitive mutation, *abi*, identifies a gene that encodes a type 2C protein phosphatase, suggesting that the phosphorylation status of the plant is important for correct ABA action. To further understand the role of ABI1 in ABA signal transduction and to identify new genes involved in ABA action, an *abi* mutant background was used to screen for mutations that further reduce ABA responsiveness in Arabidopsis. This *abi* enhancer (*abe*) screen resulted in the isolation of mutations in 6 genetic loci. Three complementation groups define the previously identified ABA response loci *ABI3*, *ABI4*, and *ABI5*. One complementation group was found to be allelic to the *CTR1* gene, a known negative regulator of ethylene signaling in Arabidopsis. Mutations at two loci identify new genes designated *ABE1* and *ABE2*. Progress in genetic characterization of *abe1* and *abe2* will be presented. To Dissect the ABA signaling pathways in Arabidopsis we used oligonucleotide-based arrays representing more than 8000 genes to determine the steady-state mRNA levels of ABA treated and non-treated “wild type”, “*abi1-1*” and “*abi1-1*/*abe1*” seedlings. The global examination of ABA regulated genes and defects in their expression in these mutant backgrounds will be presented. In addition, a number of changes in the expression of regulatory genes in *abi1-1* and *abi1-1*/*abe1* mutants from wt are observed. These changes and their significance in relation to ABA signal transduction will be presented and discussed.

271 The Arabidopsis *compact inflorescence* genes: phase-specific growth regulation and the determination of inflorescence architecture

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Flowering plants pass through a series of developmental growth phases during their lifecycle. These phases are characterized by the production of morphologically distinct vegetative and reproductive organs and by different growth patterns. Three major phases have been described: juvenile vegetative, adult vegetative, and reproductive. *compact inflorescence* (*cif*) is a novel mutant in Arabidopsis that exhibits a phase-specific phenotype. While juvenile vegetative and reproductive development in *cif* are identical to wild-type, adult vegetative organs of both the rosette and the inflorescence exhibit altered growth patterns. The most striking feature of the *cif* phenotype is a decrease in inflorescence internode length such that a cluster of flowers forms in place of the wild-type raceme. The *cif* trait is also apparent in late leaves of the rosette, and onset of the phenotype coincides with the appearance of adult morphological leaf characteristics. This correlation remains, even if the transition to adult growth is accelerated or delayed by altering the photoperiod. Reproductive organs in *cif* exhibit a wild-type anatomy, and are fully fertile. Hence, the phenotypic effects of *cif* are limited to the adult vegetative growth phase.

The *cif* mutant is distinct from other phase-associated mutants in Arabidopsis, including *hasty* and *efs*, both of which affect the timing of the phase transitions, rather than phase-specific growth patterns. Most phase mutants in maize also affect the timing of phase transitions, including *glossy15*, *viviparous8*, and the gain-of-function *Teopod* mutants. *compact inflorescence* is inherited as a two-gene trait, requiring homozygosity for the recessive mutant allele of *CIF1*, and at least one copy of the dominant *CIF2* allele.

272 The ERS1 and ETR1 members of the Arabidopsis ethylene receptor family perform an essential role in ethylene signaling

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The current model of the ethylene signaling pathway in *Arabidopsis* involves a family of five ethylene receptors, which interact with a Raf-type kinase, CTR1, to negatively regulate ethylene-response pathways through the putative ion channel EIN2. Previous studies indicated that both CTR1 loss-of-function mutants, and plants containing loss-of-function mutations in three or more ethylene receptor genes exhibit a constitutive ethylene-response phenotype (Hua and Meyerowitz, 1998). We have isolated a novel double mutant, which contains loss-of-function mutations in the ERS1 and ETR1 ethylene receptor genes. *ers1;etr1* mutants exhibit constitutive ethylene responses, but surprisingly, the defects exhibited by this double mutant are much more severe than *ctr1* or the triple loss-of-function mutants. *ers1;etr1* mutants are extremely stunted, are delayed in flowering time, and exhibit fertility and flowering defects not previously reported in any other ethylene receptor mutants. When *ers1;etr1* mutants were crossed into an EIN2-deficient background, plants exhibited *ein2*-like ethylene insensitivity, and normal flowering time, fertility and floral development were restored. One characteristic that distinguishes ERS1 and ETR1 from the other three ethylene receptor isoforms is the presence of a conserved histidine kinase domain. Experiments are underway to determine if kinase activity of ETR1 and ERS1 is essential for signaling to downstream components.

273 The regulatory roles of sterols in the development of *Arabidopsis thaliana*

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In plants, whereas the major sterols such as sitosterol and stigmasterol are structurally similar to animal cholesterol, little is known about the regulatory functions of these phytosterols. A critical role of phytosterols in embryogenesis, cell division, and cell expansion was revealed recently by the analysis of the Arabidopsis *fackel* (*fk*) mutants and cloning of the *FK* gene that encodes a C-14 sterol reductase (Genes & Dev 14: 1485-1497). Here we provide direct evidence to show that fk mutants exhibit altered response to both auxin and cytokinin. In addition, the phytosterols including the 3 novel 8,14-diene sterols accumulated in *fk* mutants are active regulators of plant growth and development. The *fk* sterols inhibit seed germination and hypocotyl elongation but enhance root growth. The *fk* sterols also considerably alter the expression of genes involved in cell expansion and division such as *TCH4, MERI-5, EXPANSIN, KORRIGAN*, and *β-TUBULIN*. In addition, the *fk* mutants can be phenocopied by treating the WT plants with a sterol C-14 reductase inhibitor, fenpropimorph. Fenpropimorh was found to impair cell expansion in a dosage-dependent manner and the inhibitory effect cannot be corrected by exogenous BL. Results of biochemical and molecular analyses indicate that fenpropimorph is a potent sterol C-14 reductase inhibitor, which can be used to assist the genetic analysis of phytosterol signaling.

274 Does jasmonate play a role in Arabidopsis leaf senescence?

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Jasmonic acid (JA) was first discovered as a leaf senescence-promoter. It has been subsequently found to be involved in many aspects of plant growth and developmental processes. However, whether JA indeed plays a role in leaf senescence remains uncertain. Here we report that JA level in senescing leaves is about five times that in non-senescing leaves in Arabidopsis, and that genes encoding enzymes for JA biosynthesis are differentially upregulated during leaf senescence in Arabidopsis; these genes include *lipoxygenase*1, *allene oxide synthase*, *allene oxide cyclase* 1 and 2, *12-oxo-phytodienoic acid reductase* 1 and 3, and *PED*1 (thiolase). Exogenous application of JA leads to precocious senescence *in planta* and in detached leaves, but has no effect on the JA-insensitive Arabidopsis mutant. These data suggest that JA play a role in leaf senescence in Arabidopsis.

275 Auxin Signal Transduction and the Cullin Gene Family

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The phytohormone auxin influences a variety of developmental and physiological processes in higher plants. Using a genetic approach, several Arabidopsis mutants were identified that showed a reduced sensitivity towards exogenously supplied auxin (*axr*, auxin resistant) or auxin transport inhibitors (*tir*, auxin transport inhibitor response). Genetic data for two of the mutants, *axr1* and *tir1*, suggested that the corresponding proteins are active in the same or overlapping pathways. Cloning of *TIR1* and *AXR1* revealed a connection between auxin signal transduction and the ubiquitin proteasome pathway. *TIR1* encodes an F-box protein, a class of proteins that are subunits of an E3 ubiquitin ligase complex Skp1-Cdc53-F-box (SCF) and a ring-finger protein RBX1. In Arabidopsis, TIR1 has been shown to interact with the Skp1-like proteins ASK1 and ASK2 and the cullin AtCUL1, a yeast Cdc53-homologue, to form an SCF-complex *in planta*. The *AXR1* gene encodes a protein that is necessary for activation of the ubiquitin-related protein RUB1. Mediated by a RUB1-conjugating enzyme called RCE1, RUB1 is transferred to a specific lysine residue of AtCUL1. Mutation of the lysine residue and overexpression of the mutated AtCUL1 protein leads to the development of pin-like flowers. Besides AtCUL1, four other cullins are present in the Arabidopsis genome that were cloned and partially analyzed. They all carry conserved domains for interaction with RBX1 and can be modified by RUB1, thus make them alternative candidates for an AXR1-dependent or related pathway. In yeast and mammals it is well established that SCF-complexes function in the regulation of multiple regulatory pathways e.g. cell cycle and carbohydrate metabolism, and a similar complex role in plants is likely. It is, however, still unclear if RUB-modification affects activity, stability or cellular localization of the SCF-complex. Thus, a detailed characterization of Arabidopsis cullins will reveal their individual role in the plant metabolic network and might give a better insight into the principle of RUB-modification.

276 Cloning of flowering genes from Pisum sativum L.

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The photoperiodic regulation of flowering in the quantitative long day plant *Pisum* is regulated by the interaction between a floral stimulus and a floral inhibitor. A physiological/genetic approach has revealed eight genes involved in flower initiation with known map location and proposed physiological function. Six of these genes, *STERILE NODES (SN)*, *PHOTOPERIOD (PPD)*, *DIE NEUTRALIS (DNE)*, *EARLY INITIATING (E)*, *HIGH RESPONSE (HR)* and *PHYTOCHROME A (PHYA)* control the production of a graft-transmissible floral inhibitor, while two other genes, *GIGAS (GI)* and *LATE FLOWERING (LF)* control the production or sensing of the floral promoter. We are using several strategies to clone pea flowering genes and pea homologues of flowering genes from *Arabidopsis* and other species, following a degenerate primer approach, PCR-select cDNA subtraction and by using differential display of total RNA. So far, we have cloned the *Arabidopsis GIGANTEA* homologue in pea. We will discuss the characteristics and the expression pattern of the *PsGIGANTEA* gene and other candidate genes in an array of pea mutants involved in flowering and/or phytochrome signaling.

277 Studying Jasmonate and Wounding Signal Transduction With Natural Variation

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Jasmonic acid (JA) mediates plant responses to pathogen infection, insect herbivory, wounding and a host of other stresses. Currently, the molecular mechanism of JA signal transduction is primarily studied via mutant analysis. This approach identified major components of the JA signal transduction pathway (COI1, JAR1, etc.). However, several elements of the pathway have yet to be elucidated.

Ja and wounding induce the accumulation of indole glucosinolates and alter the production of long chain aliphatic glucosinolates. We are studying the regulation of glucosinolates to further understand JA signal transduction. Quantifying glucosinolate concentration by HPLC analysis allows us to quantify the effects of JA signalling. A screen for natural variation in eight ecotypes identified at least two different JA signal transduction variants controlling glucosinolate biosynthesis. We are currently mapping the underlying QTLs influence these pathways. These pathways differ from the pathway that generates increased ozone sensitivity in Cvi.

278 *cryptic precocious,* a novel mutation enhancing the *FT* action in promoting floral transition

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Floral transition in *Arabidopsis* is promoted by several interacting pathways. A photoperiod-dependent pathway mediates signals from photoreceptors to a transcription factor CONSTANS, which activates downstream genes such as *FT.* Photoperiod-independent pathways, mediated by genes such as *FCA,* also activate *FT.* To elucidate pathways downstream of or in parallel with the *FT* function, we took genetic approaches. We screened EMS-mutagenized *35S::FT* plants for enhancers and suppressors of the precocious flowering phenotype. We isolated a semi-dominant enhancer which represents a novel locus, *CRYPTIC PRECOCIOUS (CRP)* and five recessive suppressors from four loci.

The *crp* mutation strongly enhanced *35S::FT* phenotype and had no rosette leaves. However, *crp* did not significantly enhance *35S::LFY* phenotype. In the absence of the *35S::FT* transgene, *crp* did not show any apparent mutant phenotype through all growth phases, and showed only slightly early-flowering phenotype in both long-day and short-day conditions. *CRP* locus was mapped to the top of chromosome 4. Genetic and physiological characterization of *crp* mutation and progress toward map-based cloning will be presented and possible roles of CRP in floral transition will be discussed.

279 *ILR2*, a novel gene involved in auxin conjugate resistance and lateral root formation

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Indole-3-acetic acid (IAA) is the most common naturally occurring auxin. Auxins are involved in virtually every aspect of plant development, making it important for plants to precisely regulate active IAA levels. One mechanism to regulate active IAA is conjugation to amino acids or sugars. These less active auxin conjugates constitute the majority of IAA found in plants. The Arabidopsis mutant *ilr2* was isolated as an IAA-leucine resistant mutant that retains wild-type sensitivity to free IAA in a screen designed to identify genes important for IAA conjugate homeostasis (Bartel & Fink, 1995, Science 268:1745-1748). ilr2 is resistant to IAA-Leu and IAA-Phe, but not to other conjugated forms of IAA. It is also defective in lateral root formation and primary root elongation. We used a map-based approach to clone the gene defective in the *ilr2* mutant, which maps to the bottom of chromosome III, between the markers nga162 and *GL1*. The *ILR2* gene codes for a novel protein that is polymorphic between Arabidopsis ecotypes. *ILR2* expression is diversely regulated in these ecotypes. Current studies directed towards analyzing the protein expression and localization will elucidate its role in IAA conjugate metabolism and lateral root formation.

280 Loss of FLOWERING LOCUS C Activity Eliminates the Late Flowering Phenotype of FRIGIDA and Autonomous Pathway Mutations but Not Responsiveness to Vernalization

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The MADS domain-containing transcription factor *FLOWERING LOCUS C (FLC)* acts as an inhibitor of flowering and is a convergence point for several pathways that regulate flowering time in Arabidopsis. In naturally occurring late flowering ecotypes, the *FRIGIDA (FRI)* gene acts to increase *FLC* levels, whereas the autonomous floral promotion pathway and vernalization act to reduce *FLC* expression. Previous work has shown that the Landsberg erecta allele of *FLC*, which is not a null allele, is able to partially suppress the late flowering phenotype of *FRIGIDA* and mutations in the autonomous pathway. In this study, using a null allele of *FLC*, we show that the late flowering phenotype of *FRIGIDA* and autonomous pathway mutants are eliminated in the absence of *FLC* activity. In addition, we have found that the down regulation of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* by *FRI* and autonomous pathway mutants also is mediated by *FLC*. Complete loss of *FLC* function, however, does not eliminate the effect of vernalization. Thus, *FRI* and the autonomous pathway may act solely to regulate *FLC* expression, whereas vernalization is able to promote flowering via *FLC*-dependent and *FLC*-independent mechanisms.

281 Genetic Analysis of Indole-3-Butyric Acid Response Mutants

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Auxins are an important class of plant hormones involved in many aspects of plant growth and development, including apical dominance, vascular development, stem elongation, lateral root initiation, phototropism, and gravitropism. Indole-3-butyric acid (IBA) is an endogenous auxin that is widely used in agricultural and commercial settings because of its strong induction of lateral roots. There are two proposed mechanisms of IBA action in plants. IBA may function solely via its conversion to the more abundant endogenous auxin, indole-3-acetic acid, by a process similar to fatty-acid β-oxidation in peroxisomes. Alternatively, IBA may act via its own signaling pathway, independent of IAA. To elucidate the mechanisms of IBA action *in vivo,*we have isolated 24 Arabidopsis IBA-response *(ibr)*mutants that are insensitive to the inhibitory effects of IBA on primary root elongation but that remain sensitive to the effects of IAA (Zolman et al., 2000, Genetics 156:1323). These mutants have been characterized and grouped into distinct phenotypic classes. Defects in growth in the absence of exogenous sucrose indicates some of the mutants are defective in enzymes acting in peroxisomal β-oxidation and, therefore, the conversion of IBA to IAA. Other mutants have normal peroxisomal function but abnormal responses to synthetic auxins and auxin transport inhibitors. In addition, a number of ethylene resistant mutants are resistant to the inhibitory effects of IBA but sensitive to IAA. These results indicate that IBA does act via its conversion to IAA in Arabidopsis, but is also distinct from IAA based on differences in transport and interactions with other hormones.

282 Identification of new mutations that alter seed ABA responsiveness in Arabidopsis using a stereoisomer of abscisic acid

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Abscisic acid (ABA) regulates numerous of physiological processes including seed dormancy and adaptive response to desiccation. Although genetic approach have been successful in the identification of genes that regulate ABA responsiveness during germination, it is not clear how ABA regulates germination at a molecular level. Recent studies on mutations that reduce ABA sensitivity (*abi3*, *abi4*, *abi5*) suggests these genes define overlapping functions that determining the sensitivity of the seed to ABA. To identify new genes involved in ABA responses during germination which may not be easily identified by conventional ABA mutant screens we have taken advantage of (-)-ABA, a stereoisomer of the natural occurring (+)-ABA isomer. The (-)-ABA isomer produces a milder physiological response compared to (+)-ABA in Arabidopsis. To date, we have identified more than 20 independent mutants defective in their germination response to (-)-ABA. Although most of the mutants appear to be less sensitive to both (+)-ABA and (-)-ABA, the sensitivity to these ABA isomers is differentially affected by various mutations. For example, the *abi4* mutations cause less sensitivity to (-)-ABA, while the *abi5* mutations appear to cause less sensitivity to (+)-ABA in seeds. A number of new loci have been identified that show differential sensitivity to the ABA stereoisomers and one of these, *CHOTO1* (*CHO1*), has been molecularly identified by map-based cloning. The *CHO1* gene appears to encode an AP2-type transcription factor.

283 Gibberellin-responsive genes during germination of Arabidopsis seeds.

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Gibberellin (GA) plays an essential role during germination of *Arabidopsis* seeds. Seeds of the severe GA-deficient mutant *ga1-3* require exogenous GA for germination. To understand GA response and its interaction with other signaling pathways during seed germination, we have analyzed GA-dependent gene expression using the oligonucleotide-based DNA microarray (GeneChip, Affymetrix) in the non-germinating *ga1-3* seeds. This system enables us to screen about 8,300 genes. Our data indicated that more than 500 genes were differentially expressed between the GA-treated and non-treated samples, which is consistent with the dramatic effects of GA on seed germination. At earlier time points (1 and 3 h after the treatment), much fewer genes were determined to be GA-responsive. We confirmed GA-responsive gene expression for some of these genes using RNA gel blot analysis and/or RT-PCR. The GA-upregulated genes include those encoding proteins involved in cell elongation, such as expansin and xyloglucan endotransglycosylase. Our results also showed that genes encoding GA biosynthesis enzymes are downregulated by GA treatment, consistent with the negative feedback inhibition of the GA biosynthesis pathway. We will examine whether these genes are expressed in response to an increase in the endogenous GA synthesis in imbibed wild-type seeds. We also plan to use these GA-responsive gene markers to understand how the GA response pathway interacts with other endogenous and environmental signals controlling seed germination.

284 Functional Analysis of the MADS-box gene *AGL31*

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We are analyzing the MADS-box gene *AGL31* for its possible function in regulating flowering time of *Arabidopsis thaliana*. *AGL31* is one member of a cluster of four tandemly-repeated MADS-box genes located at the bottom of Chromosome V, which are closely-related to each other and showing sequence homology with *FLOWERING LOCUS C* (*FLC*). Because *FLC* is a central repressor of the floral transition, we hypothesize that *AGL31* may function to regulate flowering time.

Functional analyses of *AGL31* are carried out via both gain-of-function and loss-of-function strategies. We have identified a possible knockout mutant from T-DNA mutagenesis lines and are over-expressing *AGL31* from a constitutive promoter in transgenic plants. The phenotypes of the transgenic plants will be discussed.

285 Characterizing the Role of the CKI1 Histidine Kinase in Arabidopsis thaliana

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CKI1 (cytokinin-independent) was the first non-ethylene receptor hybrid kinase discovered in *Arabidopsis*. The putative protein structure, combined with evidence that *Arabidopsis* calli overexpressing CKI1 exhibit a "cytokinin-independent" phenotype (1), led to the idea that CKI1 is involved in cytokinin signaling, perhaps acting as a cytokinin receptor. To test the function of CKI1 *in planta*, we have used a reverse genetic approach to identify plants with a T-DNA insertion in CKI1. Two independent alleles were identified, which produce the same effect on plant development. Analyses of populations segregating the *cki1-1* (within the third exon) or *cki1-3* (600 bases upstream of the ATG) T-DNA insertion allele failed to reveal any homozygous cki1 plants, suggesting that the homozygous condition is lethal. Based on data from reciprocal crosses, as well as a close examination of developing siliques, we suggest that CKI1 function is required for female gametophyte development. A closer examination of the female gametophyte defects, as well as investigations of where and when CKI1 is expressed at other stages in *Arabidopsis* development, are currently underway. Our work with CKI1 mutants indicates that signal transduction via a His/Asp phosphorelay system may play an important and previously unsuspected role in female gametophyte development in *Arabidopsis.* 1. Kakimoto, T. (1996) Science274, 982-985. This work was supported by an NSF graduate research fellowship.

286 Analysis of degradation of proteins important for a plant hormone response

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The goals of this study are to determine the degradation rates of proteins important in the auxin response. We have previously shown that a full-length Aux/IAA protein from *Pisum sativum*, PSIAA6, acts as a transferable degradation signal when fused to the reporter enzyme, firefly luciferase (LUC) (Worley *et al*., 2000). We report further on defining this degradation signal. The major cis-acting signal does not include PSIAA6 amino acid residues 71-179, which contain domains III and IV conserved among members of the Aux/IAA family of proteins. In addition, we report that an additional Aux/IAA protein from *Arabidopsis thaliana*, IAA1, when expressed as a fusion protein with LUC, acts as a transferable degradation signal. Its half-life, as measured by LUC activity in cycloheximide chase experiments on transgenic seedlings, is statistically no different than that observed for PSIAA6::LUC. To determine the protease machinery involved in Aux/IAA degradation, we tested the ability of proteasome inhibitors to affect the accumulation of an Aux/IAA::LUC fusion protein in vivo, and observed a 3 to 8-fold steady-state increase in the Aux/IAA::LUC fusions tested.

Worley, C.K., Zenser, N., Ramos, J., Rouse, D., Leyser, O., Theologis, A., and Callis, J. (2000). Degradation of Aux/IAA proteins is essential for normal auxin signaling. Plant J. 21, 553-562

287 IAR1, a novel membrane protein involved in auxin homeostasis

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The most common naturally occuring form of the plant hormone auxin is indole-3-acetic acid, or IAA. In Arabidopsis, up to 95% of the IAA pool is found conjugated to small molecules such as amino acids. Although these conjugates likely play an important role in the regulation of IAA levels within the plant, the genes and enzymes involved in IAA conjugate metabolism are not yet well understood. We are investigating auxin homeostasis by isolating Arabidopsis mutants that respond abnormally to IAA-amino acid conjugates. We identified a mutant, iar1, that is resistant to the inhibitory effects of multiple IAA-amino acid conjugates on root elongation but that remains sensitive to free IAA. The IAR1 gene encodes a protein with numerous transmembrane domains and several histidine-rich regions (Lasswell et al., 2000, Plant Cell 12:2395-2408). The IAR1 protein has homologs in other organisms, including Drosophila, C. elegans, and mammals, and is similar in molecular structure to the ZIP family of metal transporters from Arabidopsis and yeast. We are currently determining the subcellular localization of IAR1 with a microsomal two-phase partition system. In addition, we have isolated mutants that enhance or suppress the IAA-amino acid conjugate resistant phenotype of iar1 roots. The identification of proteins that physically or genetically interact with IAR1 will aid in deciphering the role of IAR1 in IAA-conjugate metabolism.

288 Function of a MAP kinase pathway in plant HR-like cell death is salicylic acid-independent

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Mitogen-activated protein kinase (MAPK) cascades play important roles in transducing extracellular signals into cellular responses in yeast and animals. A MAPK cascade is composed of three interlinked kinases including a MAPK, a MAPK kinase (MAPKK or MEK), and a MAPKK kinase (MAPKKK). An increasing body of evidence implicated SIPK and WIPK, two tobacco MAPKs, as well as their orthologs in other species in plant defense responses. Recently, $NtMEK_2$, a tobacco MAPKK was identified as the upstream kinase for both SIPK and WIPK. Using a gain-of-function approach, we found that the long-lasting activation of SIPK and WIPK by $NtMEK_2^{DD}$, an active $NtMEK_2$mutant induces HR-like cell death in tobacco. In addition, we discovered that $AtMEK_4$ and $AtMEK_5$, two Aabidopsis MAPKKs that shares about 70% identity in amino acid sequence with tobacco NtMEK2 are functionally interchangeable with NtMEK2. Expression of $NtMEK_2^{DD}$, $AtMEK_4^{DD}$ or $AtMEK_5^{DD}$ in Arabidopsis leads to the activation of endogenous $AtMPK_6$ and $AtMPK_3$ and cell death. To examine if salicylic acid (SA), an important secondary signaling compound in plant defense response is involved, we crossed $NtMEK_2^{DD}$ and NahG transgenic Arabidopsis plants. NahG encodes the SA-metabolizing enzyme salicylate hydroxylase. As a result, the SA-dependent responses in such transgenic plants are compromised. Comparison of the phenotype of $NtMEK_2^{DD}$ x NahG and $NtMEK2^{DD}$ x wild type plants indicated that SA is not required for $NtMEK_2^{DD}$-induced cell death.

289 Pathogen defense- and senescence-related gene expression is positively and negatively modulated by a WRKY transcription factor

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In *Arabidopsis*, WRKY factors comprise a large multigene family of plant-specific transcriptional regulators controlling several types of stress responses. We present data showing that expression of one representative, *AtWRKY6*, is influenced by several external and internal signals often involved in triggering plant defense reactions and senescence processes. Furthermore, protein truncations as well as gain-of-function studies revealed that a novel type of nuclear localization signal (NLS) mediates nuclear localization of *At*WRKY6.To understand its regulatory role, we identified several potential *At*WRKY6 target genes by the use of an *Atwrky6* null-mutant and an *AtWRKY6* overexpression line. One such candidate gene, *SIRK*, encodes a receptor-like protein kinase, whose expression is strongly induced only during leaf senescence. The transcriptional activation of *SIRK* is *At*WRKY6-dependent. Senescing leaves of *Atwrky6* null-mutants showed a drastic reduction in *SIRK* transcript levels, whereas these levels were clearly elevated in green leaves of the *AtWRKY6* overexpressor lines. Furthermore, *At*WRKY6 induced *SIRK* gene promoter activity *in vivo*. Similarly, the promoter activity of the pathogenesis-related *PR1* gene was also positively influenced by *At*WRKY6. In addition to its function as a trans-activator, *At*WRKY6 also can act as a negative regulator. In particular, *At*WRKY6 suppressed *AtWRKY6* gene promoter activity indicating negative autoregulation. *At*WRKY6 also repressed the promoter activity of a closely related WRKY gene family member. Taken together, *At*WRKY6 acts as a bifunctional transcriptional regulator controlling both pathogen defense and senescence-related gene expression.

290 A gain-of-function mutation in *IAA28*, an auxin-repressed gene, suppresses lateral root development and alters auxin responses

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Auxins are an important class of plant hormones that are implicated in most aspects of plant development, and thus influence the overall size and shape of a plant. While the signal transduction pathways that sense and respond to auxin remain mysterious, a number of genes undergo dramatic transcriptional alterations in response to auxin. For example, the *Aux/IAA* genes were originally isolated based on strong and rapid transcriptional up-regulation induced by auxin. *Aux/IAA* genes are primary response genes whose products are thought to regulate auxin-responsive transcription. We cloned a new member of the *Aux/IAA* gene family, *IAA28*, based on the abnormal auxin responses and unusual auxin-related adult phenotypes of a gain-of-function mutant, including decreased apical dominance and extremely reduced lateral root formation (Rogg et al. (2001) Plant Cell 13: 465-480). In addition to auxin, the *iaa28-1* mutant is also resistant to inhibition of root elongation by cytokinin and ethylene, but responds normally to other phytohormones. Northern analysis and promoter-GUS fusions demonstrate that *IAA28* is strongly expressed in roots. *IAA28* transcription is up-regulated in response to cycloheximide, suggesting it is a primary response gene. However, *IAA28* transcription decreases upon auxin treatment, a response that differs from all other characterized members of the *Aux/IAA* gene family. Experiments with the auxin-inducible BA-GUS construct suggest that IAA28 is a repressor of auxin-induced transcription. *IAA28* may encode a transcriptional repressor that functions to regulate the expression of genes that promote lateral root initiation in response to auxin signals. We are testing this model by monitoring changes in IAA28 protein accumulation in response to various factors, identifying genetic suppressors of *iaa28-1* and analyzing global gene expression in the *iaa28-1* mutant.

291 FLC protein levels determine the magnitude of vernalization-responsive late-flowering.

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The MADS-box protein encoded by *FLOWERING LOCUS* C (*FLC*) is a repressor of flowering that is down regulated by vernalization. The level of FLC protein directly correlates with the magnitude of vernalization-responsive late-flowering in a range of Arabidopsis ecotypes and mutants. *FLC* antisense constructs in the *fca-1* background severely reduced the late-flowering *fca-1* phenotype, indicating that in *fca-1* and probably other autonomous pathway mutants the late-flowering phenotype is due to upregulation of *FLC* (Sheldon *et al*. (2000) *PNAS* **97,** 3753-3758). The vernalization-induced decrease and genetically controlled changes in the level of FLC protein parallel that of the *FLC* transcript (Sheldon *et al.,* 2000), emphasizing that control is at the level of transcription or transcript stability. We show that the epistatic groupings of *fca*/*fy* and *fve*/*fpa*, based on their effects on flowering-time (Koornneef *et al*. (1998) *Genetics* **148,** 885-892), are consistent with their effects on *FLC* transcript and protein levels. We have also examined FLC protein levels in a range of photoperiod and autonomous pathway double mutants (Koornneef *et al.,* 1998) and show that FLC levels account for an important component of the late-flowering phenotype of some of the double mutant lines.

The upregulation of *FLC* transcript and protein in vernalization-responsive mutants in the Landsberg *erecta* (L*er*) ecotype occurs to a much lesser extent in root than in aerial tissue; this is in contrast to other ecotypes in which FLC protein levels are similar in both root and aerial tissues, suggesting that different *FLC* regulatory pathways may operate in root and aerial tissue, and that L*er* differs from other ecotypes in one or more loci involved in control of root specific expression of *FLC*.

In C24 plants with high FLC levels, flowering in the absence of vernalization can occur without a decrease in FLC protein levels. Another MADS-box gene, *SOC1* (*AGL20*) is a promoter of flowering. *SOC1* is negatively regulated by FLC, however *SOC1* levels can increase at flowering without an associated decrease in FLC protein. This suggests that FLC mediated inhibition of *SOC1* expression can be circumvented via another pathway.

292 Interactions of the COP9 signalosome and protein degradation pathways

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The COP9 signalosome is an evolutionary conserved multi-protein complex of unknown function that acts as a negative regulator of photomorphogenic seedling development in Arabidopsis. We have recently found that plants with reduced COP9 signalosome levels show decreased auxin-response similar to loss-of-function mutants of the E3 ubiquitin ligase SCF^{TIR1}. Furthermore, we have evidence for a physical interaction between the COP9 signalosome and SCF^{TIR1}. AUX/IAA proteins are candidate substrates of SCF^{TIR1} and we found that AUX/IAA proteins are inefficiently degraded in COP9 signalosome mutants. Thus, the COP9 signalosome may play an important role in mediating auxin-response via interactions with SCF^{TIR1}. A possible role of the COP9 signalosome in mediating other E3 ubiquitin ligase-requiring processes will be discussed.

293 SLEEPY1, a GA-response gene of Arabidopsis.

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sleepy1 (sly1) is a recessive GA-insensitive mutant displaying the full spectrum of phenotypes expected in a GA mutant. sly1 is a dark green severe dwarf with reduced fertility and increased seed dormancy. Interestingly, the seed dormancy phenotype of sly1 has varying degrees of expressivity. Some seed lots of sly1 will show high levels of germination, while others show only 10-20% germination. This phenotype is subject both to afterripening and secondary dormancy. Those seed lots that germinate well have increased sensitivity to ABA compared to wild type. For example, a seed lot of sly1-10 giving 97% germination in the absence of hormone, gives 17% germination on 0.3 microM ABA and 0% germination on 0.6 microM ABA. Currently, we are performing ultrastructural analyses of hyper-dormant sly1 seed to see if this phenotype correlates with any apparent anatomical characteristics. In an effort to learn where SLY1 fits into the GA signal transduction pathway, we are performing a double mutant analysis of sly1 versus rga-2 and spy1-4. Although rga-2 rescues dwarfism in the GA biosynthetic mutant ga1-3, it fails to rescue dwarfism in sly1-10. One interpretation is that SLY1 acts downstream of the RGA transcription factor. In contrast, spy1-4 partially rescues the dwarfism of sly1-10. This may point to an additive effect of these mutants. We are in the process of cloning SLY1 by map-based cloning.

294 Dissecting auxin signalling: Isolation of genetic interactors with *AXR3*

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AXR3/IAA17 is a member of the Aux/IAA family of early auxin-induced transcriptional regulators from *Arabidopsis*. Aux/IAA proteins are extremely unstable and share 4 highly conserved domains. Sequence analysis suggests that domains I and II mediate protein-protein interactions. Furthermore, semi-dominant mutations in domain II of *AXR3* result in an increased magnitude of auxin responses associated with higher stability of the protein. To find novel members of the auxin signalling pathway, we are searching for genes that interact with *AXR3*. We have isolated a second-site partial suppresser of the *axr3-1* gain of function phenotype, *pax1-1*. The *pax1-1* single mutant is recessive to wild type and shows pleiotropic phenotypes suggestive of altered auxin and gibberellin responses. *pax1-1* represses expression of an AXR3 promoter::GUS reporter in transgenic *Arabidopsis*. Double mutant analysis suggests that *PAX1* also interacts with other members of the *Aux/IAA* family. In addition, we have isolated proteins that appear to interact with domain I and/or II of *AXR3* in the yeast two-hybrid system.

295 DAG1 and DAG2: two transcription factors involved in seed germination in Arabidopsis.

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The Dof proteins are transcription factors widely distributed within the plant kingdom, characterized by a strongly conserved zinc finger domain. The conservation of this domain, coupled with the presence of the Dof transcription factors in all plants, suggests a crucial role of these proteins in regulating functions typical of plants. By means of a reverse genetics approach, we demonstrated that the Arabidopsis Dof protein DAG1 is involved in the maternal control of seed dormancy and germination (Papi et al. 2000, Gen. & Dev. 1). The phytochrome–mediated pathway for seed germination is still required in *dag1-1* mutant seeds as a far-red light pulse is inhibitory. We subsequently identified a T-DNA insertion line in another uncharacterized gene of the Arabidopsis Dof family, and the corresponding gene was denominated *DAG2* for its sequence similarity with *DAG1* (76,2% aminoacidic identity). *dag2-1* was shown to be a knock-about mutant, as the N-terminus of DAG2 is expressed in translational fusion with the GUS gene. Both DAG1 and DAG2 show a tissue-specific expression localized in the vascular tissue in the whole plant. The *dag2-1* mutant line is less sensitive to all the parameters that promote germination, such as vernalization, light and GAs, thus showing an opposite phenotype as compared to *dag1-1* .DAG1 and DAG2 might thus be involved in interacting pathways or even regulate with opposite roles the same gene(s), based on their overlapping expression patterns and on the opposite phenotype of the respective mutants. We have performed an ESTs-microarray screening to identify genes that are differentially expressed in the *dag1* and *dag2* mutants as compared to the wild type, with the aim of identifying the targets of DAG1 and DAG2. This will allow to shed light on the mechanism of red light–induced seed germination in Arabidopsis and to establish whether DAG2 and DAG1 have opposite effects in controlling the same regulatory circuits. In addition, ongoing transient expression experiments by particle gun transformation will assess the respective regulatory roles of the DAG1 and DAG2 proteins.

296 The Isolation and Characterisation of *sar1* and Other Suppressors of *axr1*.

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The auxin resistant mutant *axr1* displays defects in apical dominance, lateral branching of both roots and shoots and hypocotyl elongation. These and other phenotypes such as reduced accumulation of the SAUR and IAA transcripts are consistent with a defect in auxin response.The *AXR1* gene encodes the N-terminal half of a RUB-activating enzyme, analogous to the E1 ubiquitin-activating enzyme (Del Pozo et al. Science 1998. 280: 1760-1763). AXR1 functions as a hetero-dimer with ECR1, an E1 C-terminal like protein, to activate RUB, a ubiquitin-like protein. RUB is conjugated to a cullin AtCUL1, in an AXR1-dependent manner. AtCUL1 is part of an E3-ubiquitin ligase SCF complex along with SKP1, RBX1 and an F-box protein TIR1 (Gray et al 1999. Genes Dev. 13: 1678-1691). This complex may function to degrade repressors of auxin action.

The *sar1* (*suppressor of axr1*) mutant was isolated as suppressing the auxin resistant root defect of *axr1* and has been demonstrated to suppress virtually every aspect of the *axr1* phenotype (Cernac et al. 1997 Dev. 124: 1583-1591). Genetic studies indicate that *sar1* may function downstream of *AXR1*, suggesting that SAR1 may be a target of the pathway. Interestingly, *sar1* does not suppress the *tir1* mutant indicating that it is unlikely to be a target of the TIR1 SCF complex.It may however be a target of one of the TIR1-related proteins or it may have some other function in the pathway. A positional cloning strategy is being used to address the question of the role of SAR1 in this pathway. *Sar1* maps to an approximately 100 kb region on chromosome 1 between m253 and mi423a. A cosmid contig is currently being assembled through this region.

Seedlings grown in the light at high temperature exhibit dramatic hypocotyl elongation, a growth response which is proposed to be associated with increased flux through the auxin response pathway and which is absent in the *axr1* mutant (Gray and Estelle 1998. PNAS 95:7197-7202). A screen to identify suppressors of this *axr1* hypocotyl defect identified a number of new mutants. Data will be presented on both the recessive *sar3* and semi-dominant *sar6* mutants. Both mutants map close to the bottom of chromosome 1.

297 Genetic studies of IAA supersensitivity

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Auxins affect many aspects of plant growth and development through regulation of gene expression and cell growth. It is therefore essential for plants to temporally and spatially regulate the amount of active auxin. The known auxin resistant mutants suggest that auxin homeostasis is dependent, at least in part, on negative regulation. We designed a screen that led to the identification of two Arabidopsis auxin supersensitive (*axs*) mutants defective in this regulation. The *axs* mutants have an increased number of lateral roots when grown at 28º, a condition known to induce auxin accumulation. Both mutants are deficient in root waving and curling, which are associated with auxin transport. *axs2* is resistant to root elongation and lateral root inhibition in response to the auxin transport inhibitors NPA and TIBA. Using recombination mapping, we have localized the gene defective in *axs2* to the bottom of chromosome I, between the markers nga280 and nga111. In addition, we are using reverse genetics to analyze candidate negative regulators of auxin homeostasis in Arabidopsis.

298 BRI1 Homologues Are Potential BR Receptors

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BRI1 is a member of the leucine-rich-repeat (LRR) receptor-like kinase (RLK) family and functions as a component of a plasma membrane brassinosteroid (BR) receptor. Database searches identified three Arabidopsis genes encoding proteins that display significant sequence identity with the BRI1 protein and contain the characteristic 70 amino acids island domain, which is critical for the perception of BR signals by BRI1. Expression of two of the three genes under the control of the BRI1 promoter rescues *bri1* mutant phenotypes, indicating that these two genes encode functional BRI1 homologues. Promoter-GUS-fusion analyses reveal that all three genes are mainly expressed in the vascular tissues of various plant organs, raising an interesting possibility that all three proteins might mediate BR signaling involved in vascular development. To test this idea, we screened several collections of Arabidopsis T-DNA insertional lines and identified a knockout mutant for each of the three genes. Careful examination of these single mutants did not reveal any noticeable morphological change. Such an observation is not surprising since these genes share a very high degree of sequence identity and similar expression pattern and might well be performing redundant functions in a specific BR-regulated process. Double and triple knockout lines are currently being constructed to reveal their functions in plant development.

299 Dynamic Changes in the Expression Levels of Ethylene Receptors from Arabidopsis

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Hormone responses can be modulated by varying receptor levels as well as by varying hormone levels. We examined expression levels of ethylene receptors in Arabidopsis using DNA microarrays. Expression of the ethylene-receptors *ETR2*, *ERS1*, and *ERS2* was stimulated in the constitutive ethylene-response mutant *ctr1-2* compared to the ethylene-insensitive mutant *etr1-1*. Exposure of plants to salt-stress resulted in reduced expression of the ethylene receptors *ETR1*, *ETR2*, and *ERS1*. Western blot analysis was used to confirm changes in ETR1 and ETR2 at the protein level. The effect of ethylene-pathway mutants upon expression of the ethylene-receptor ETR1 was also examined. For this purpose, ETR1 protein levels were quantified in mutant backgrounds containing receptor loss-of-function mutations, ethylene-insensitive mutations and constitutive ethylene-response mutations. Our results indicate that expression of the ethylene receptors is dynamically regulated in plants and that this could affect signaling through the ethylene signal-transduction pathway. Our results also suggest that ethylene signal-transduction plays a role in the salt-stress response of plants.

300 RNA directed promoter methylation and transcriptional gene silencing in *Arabidopsis thaliana*

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Homology-dependent gene silencing can occur transcriptionally or post-transcriptionally, affecting either mRNA synthesis in the nucleus or mRNA stability in the cytoplasm. Because of the sequence specificity of both processes, nucleic acid interactions such as DNA-DNA or RNA-DNA pairing for transcriptional and RNA-RNA pairing for post-transcriptional gene silencing are likely to be involved. Double stranded RNA is known to induce a post-transcriptional gene silencing process, termed RNAi, in diverse organisms. Recent studies (1,2) showed that double stranded RNA containing promoter sequences triggers transcriptional gene silencing of a non-linked homologous target promoter in tobacco and *Arabidopsis*. This process is accompanied by *de novo* methylation of the target promoter and the presence of small RNAs approximately 23 nucleotides in length which are produced by cleavage of the promoter double stranded RNA. RNA hairpins transcribed from inverted DNA repeats were the most effective *trans*-acting silencing signals. A similar approach was used to selectively silence and methylate a single member of an endogenous gene family with very conserved open reading frames but distinct promoter regions.

Different mutant alleles of genes known to be involved in either transcriptional or post-transcriptional gene silencing in *Arabidopsis* have been crossed into our silencing system. Conclusions from these studies will be discussed. A mutant screen for target promoter reactivation in the presence of the *trans*-silencing signal is in progress.

This work has been supported by grants from the Austrian “Fonds zur Förderung der wissenschaftlichen Forschung” (Z21-MED).

1. Mette MF et al. (1999) EMBO J. 18: 241-248 2. Mette MF et al. (2000) EMBO J. 19: 5194-5201

301 Identification of a gene that controls non-CG methylation in Arabidopsis

Lisa Bartee and Judith Bender

Johns Hopkins School of Public Health

We are using the methylated endogenous phosphoribosylanthranilate isomerase (*PAI*) genes in the Arabidopsis strain WS to identify trans-acting factors that affect *PAI* methylation. In the WS strain of Arabidopsis there are four genes encoding the PAI enzyme: *PAI1* and *PAI4* arranged as a tail-to-tail inverted repeat, and singlet *PAI2* and *PAI3* genes. These four genes are methylated over their regions of DNA identity at both CG and non-CG cytosines. Of the four WS *PAI* genes, only the *PAI1* and *PAI2* genes encode functional PAI enzyme. The *PAI2* gene is silenced by methylation, but the *PAI1* gene is expressed despite methylation, accounting for the wild type WS phenotype. To facilitate genetic analysis of this system, we isolated a WS mutant with a missense mutation in the coding sequence of the expressed *PAI1* gene. The mutant displays a strong blue fluorescent phenotype as well as decreased size and fertility resulting from the drastic decrease of PAI enzyme activity, but the four *PAI* genes remain methylated and the structure of the inverted repeat is not impaired. In this *pai1* reporter strain, any mutations that act to reduce the silencing of the functional *PAI2* gene can be easily identified by a reduced-fluorescence phenotype.

A mutant recovered from this screening strategy displayed greatly decreased seedling fluorescence and moderately decreased adult plant fluorescence as well as increased size and fertility. Analysis of methylation levels on the *PAI* genes by Southern blot and bisulfite genomic sequencing showed a substantial decrease in non-CG methylation. Since the *PAI2* promoter contains mostly non-CG cytosines, loss of their methylation accounts for the reactivation of *PAI2* expression and the reduction of PAI-deficient phenotypes. A decrease in non-CG methylation was also detected at centromeric and rDNA repeat sequences, consistent with the pattern observed for the *PAI* genes. The mutant locus was cloned by positional methods, and characterization of the gene product will be presented.

302 SLO: A FACTOR DEFINING AN ETHYLENE PATHWAY IN PARALLEL TO ETR1 AND EIN2?

S. Bertrand, C.Zhang, J.Smalle, M.Haeman, E.Fostier, and D.Van Der Straeten

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SLO : A FACTOR DEFINING AN ETHYLENE PATHWAY IN PARALLEL TO ETR1 AND EIN2? S. Bertrand, C. Zhang, J. Smalle, M. Haegman, E. Fostier, and D. Van Der Straeten Vakgroep Moleculaire Genetica en Departement Plantengenetica, Vlaams Interuniversitair Instituut voor Biotechnologie, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium Ethylene is a unique gaseous signalling molecule, with a simple molecular structure. It has multiple effects in development and in response to numerous forms of stress. By using the triple-response assay for isolation of ethylene mutants, the ethylene-signalling pathway has already been well-described (Chang et al., 1999). However, several facts suggest the existence of additional components in ethylene signalling. To identify additional components in ethylene signalling, novel screening methods were designed (Smalle and Van Der Straeten, 1997; Smalle et al., 1997). Consequently, slo, a leaf emergence mutant defining a new locus involved in ethylene-regulated development was isolated. The slo mutation causes a reduction in cell expansion, and a general delay in phase transitions. Thus, when compared to wild type and relative to the preceding phase transition, slo is retarded in germination, leaf emergence, flowering, and senescence. Slo seedlings were less sensitive to the inhibitory effect of ACC on leaf expansion. On high concentrations of ACC, not only wild-type plants but also the ethylene-insensitive mutants etr1-3 and ein2-1 showed a strongly reduced leaf and root expansion, whereas sloetr1-3 and sloein2-1 double mutants remained virtually unaffected. This enhancement effect reflects an increased insensitivity to ethylene as demonstrated both by using 1-methylcyclopropene, an inhibitor of ethylene action, and by RNA gel blot analysis of ethylene-induced genes in slo, etr1-3, ein2-1, and in double-mutant backgrounds. We propose that SLO acts in a pathway parallel to ETR1 and EIN2 in mediating effects of ethylene on Arabidopsis development. The SLO gene cloning is in progress. *et alet alsloslosloSloetr1-3ein2-1sloetr1-3sloein2-1slo, etr1-3, ein2-1*

303 Isolation and characterization of proteins required for chromosome condensation and sister chromatid cohesion during meiosis and mitosis

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Proper condensation, pairing and segregation of chromosomes are essential events in mitosis and meiosis. Cohesins are proteins that are required for sister chromatid cohesion. Two different cohesin proteins have been identified in yeast. One is essential for meiosis while the second functions mainly during mitosis. Much less is known about the proteins required for chromosome condensation and sister chromatid cohesion in higher eukaryotes, in particular plants. Studies in our laboratory are designed to identify proteins required for chromosome condensation and sister chromatid cohesion and determine how they function. Forward and reverse genetic experiments are being used to isolate mutants of Arabidopsis defective in chromosome condensation and cohesin. Arabidopsis contains four cohesin genes (SYN1-4). SYN1 is required for meiosis. Syn1 plants are male- and female-sterile, exhibiting defects in chromosome condensation and pairing at leptonema, followed by fragmentation of chromosomes. Protein localization studies have shown that SYN1 localizes to meiotic chromosomes from interphase to metaphase I; it is not detectable after metaphase I or during mitosis. SYN2, SYN3 and SYN4 are expressed throughout the plant with highest transcript levels present in meristematic cells. In contrast to the syn1 mutation, mutations in syn2 have no detectable phenotype. SYN2 is detectable during interphase and early prophase of cell cultures, but absent from late prophase and metaphase cells. Results from additional mutational and protein localization studies will be presented.

304 Chromatin modifications and transgene silencing

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A widely accepted hypothesis is that cytosine methylation signals the recruitment of histone deacetylases which then modify chromatin to silence genes. This linear pathway is supported by the physical association of methyl binding proteins with histone deacetylases. Also consistent with the model is the derepression of silenced rRNA genes in interspecific hybrids using either aza-deoxycytidine (aza-dC; an inhibitor of cytosine methylation) or Trichostatin A (TSA; an inhibitor of certain histone deacetylases), with no additive or synergistic affect of using both chemicals. However, it is not known if the model is generally true. We screened A. thaliana GFP enhancer-trap lines to identify lines containing silenced transgenes that could be derepressed by treatment with aza-dC or TSA. Of 23 lines identified (out of approximately 750 screened), 18 showed release of GFP silencing by aza-dC but not by TSA. In addition to the lack of redundancy, a surprising finding is that in aza-dC-responsive lines, TSA tends to antagonize the derepressing effects of aza-dC. A. thaliana transgenic lines carrying silenced GFP genes that respond to aza-dC were screened for mutations that can confer constitutive transgene expression. Several mutants have been identified and at least one displays a strong ddm (decreased DNA methylation) phenotype. Interestingly, TSA treatment silences the otherwise constitutive GFP expression in this mutant. These data suggest that cytosine methylation and histone deacetylation are not necessarily linked in simple, linear gene silencing pathways.

305 A suppressor screen of *ein2-44* to identify genes regulating ABA sensitivity in seed germination

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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 genetic pathway of this response (i.e. screening for mutants that are either ABA-insensitive (*abi*) or have an enhanced response to ABA (*era*) in germination). To date however, relatively few genes have been identified using these approaches. In an attempt to identify new genes, we have initiated a suppressor screen in an *ein2* background. The *ein2* mutant was first isolated by its insensitivity to ethylene in the seedling "triple response", and more recently a new allele of *ein2* (*ein2-44*, formerly *era3-1*) was selected for its enhanced responsiveness to ABA at germination (Ghassemian et. al. 2000). We discuss the characterization of mutants from this suppressor screen of *ein2-44*.

306 A Novel Homeobox Gene in *Arabidopsis thaliana*

Gillian Fozzard and Keith Lindsey
University of Durham

Homeobox genes were first discovered more than 70 years ago when mutations which cause disturbance of body plan in the fruitfly, *Drosophila melanogaster* were identified. Since then, homeobox genes have been found in a huge diversity of animals, from earthworms to mammals, where they are involved in body plan organisation and patterning. More recently, homeobox genes have been found in plants. Here their role is less clear cut and they appear to have roles in other developmental and response processes as well as in patterning.

These genes encode transcription factors which contain a highly conserved region known as the homeobox within their DNA sequence. The homeobox encodes a DNA binding domain of 60 amino acids which enables the homeodomain protein to regulate transcription of the target gene.

Homeobox genes have now been isolated from various plant species, including the model plant *Arabidopsis thaliana*. These plant homeobox genes can be divided into five families based on the sequence of the homeobox itself and of other conserved domains.

This work describes the isolation of a novel plant homeobox gene from *Arabidopsis thaliana* using a 3' Random Amplification of cDNA Ends (RACE) approach.

307 Functional analysis of a senescence-associated acyl hydrolase gene in Arabidopsis

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Leaf senescence is a genetically controlled developmental process that is believed to be driven by expression of senescence-associated genes (*SAG*s). Thus far, only a limited number of *SAG*s have been isolated, and their functions are unknown. In order to fully understand the biochemical components and molecular mechanisms underlying leaf senescence, it is necessary to identify and characterize additional *SAG*s. We have employed enhancer trap strategy to identify *SAG*s in Arabidopsis. By screening 1300 Arabidopsis enhancer trap line, 147 senescence enhancer lines (Sels) were found to show expression of a reporter gene (*GUS*) in senescing leaves but not in non-senescing ones. Here we report the cloning and functional characterization of *SAG101* from one of the enhancer trap lines. Recombinant *SAG101* fusion protein overexpressed in *E. coli* possesses acyl hydrolase activity. Antisense RNA interference of *SAG101* delayed the onset of leaf senescence whereas inducible overexpression of this gene caused precocious leaf senescence in transgenic Arabidopsis. These data suggest that *SAG101* play a role in leaf senescence.

308 RNA mediated transcriptional gene silencing of a seed specific promoter in *Arabidopsis thaliana*

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Double-stranded RNAs (dsRNA) that contain promoter sequences can trigger transcriptional inactivation in trans of gene driven by a homologous promoter (1). Small RNAs ~23 nucleotides derived from dsRNA are implicated in this dsRNA mediated transcriptional gene silencing phenomenon and in *de novo* methylation of the target promoter, but the molecular mechanism(s) is still unclear. To investigate this silencing machinery, a tissue specific promoter system was developed. In this system, several new aspects of the silencing machinery are expected to arise in silenced tissue vs. non-silenced tissue in one plant. A seed-specific promoter (the α' promoter, which drives expression of the gene encoding the α' subunit of the β-conglycinin protein in soy bean) was chosen as a target promoter. Transgenic target lines were established for two different reporter genes (GUS and GFP), which were fused to the α' promoter. Appropriate homozygous target lines were supertransformed with a silencer construct designed to transcribe an inverted repeat of the target promoter with short spacer sequences between the repeats by either the 35S promoter (constitutive promoter) or the vicilin promoter (seed-specific promoter). Drastic reductions of reporter gene activity were observed in all target-silencer combinations tested. Results of molecular analyses will be presented.

(1) Mette, M. F. *et al*. (2000), EMBO J. 19: 5194-5201.

309 The XRN-family of 5’-3' Exoribonucleases in *Arabidopsis thaliana*

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We are studying the XRN-family in Arabidopsis with emphasis on the potential function of these enzymes in cytoplasmic mRNA degradation. In Arabidopsis, the XRN-family consists of three enzymes, AtXRN2, AtXRN3 and AtXRN4, which are orthologs of the yeast nuclear protein Xrn2p/Rat1p. Interestingly, the Arabidopsis genome does not encode any orthologs of Xrn1p, the cytoplasmic enzyme responsible for the decay of the majority of mRNAs in yeast. An additional difference between plants and yeast is that in yeast, insertion of poly(G) tracts into mRNAs results in the accumulation of poly(G)-stabilized mRNA decay intermediates, while such intermediates have not been observed in plant cells. Poly(G)-stabilized mRNA decay intermediates accumulate in yeast since Xrn1p is blocked by poly(G) tracts and cannot efficiently degrade the RNA 3' of the poly(G) tract. The AtXRNs are also blocked by poly(G) tracts, indicating that the absence of poly(G)-stabilized mRNA decay intermediates in plant cells is unlikely due to an AtXRN progressing directly through the poly(G) tract. To further examine the potential role of the AtXRNs in mRNA degradation their intracellular locations were investigated. Localization studies of AtXRN-GFP fusion proteins revealed that AtXRN2 and AtXRN3 are targeted to the nucleus, while AtXRN4 accumulates in the cytoplasm. AtXRN4’s cytoplasmic location indicates that it might have a function in mRNA degradation. Plants bearing T-DNA insertion mutations in the *AtXRN4* gene have been identified. To investigate AtXRN4’s potential role in cytoplasmic mRNA degradation, cDNA microarrays are being used to compare mRNA degradation in xrn4 mutants to mRNA degradation in wildtype. Funding provided by: DOE, USDA and NSF

310 Autonomous Minichromosomes in Arabidopsis

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Arabidopsis has the most complete, contiguous nucleotide sequence of centromeric DNA in a higher eukaryote, making it an excellent model organism for centromere studies. All five Arabidopsis centromeres have a similar organization: large tracts of 180 bp repeat sequences flanked by repetitive DNA that is, surprisingly, interspersed with unique DNA, including expressed genes. To determine which DNA elements are important for function, we have begun to systematically test fragments from centromere 2 by building minichromomes and determining meiotic and mitotic stability based on color assays. Constructs are being made with or without telomeres. Constructs with telomeres are monitored for stability and autonomy, while constructs without telomeres are tested for end healing and autonomy or integration into host chromosomes and possible dicentric formation. As a benchmark for stability we have also quantitated fidelity of the five natural chromosomes.

311 A mitochondria-targeted homologue of E.coli RecA in Arabidopsis thaliana

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Higher plant mitochondrial genomes contain repeated sequences that are proposed to be sites for homologous intragenomic recombination. Although there are numerous reports attributing the fluidity of plant mitochondrial genomes to recombination activity, there is little biochemical or genetic evidence for the existence of a definitive homologous recombination mechanism in the mitochondria of plants. Here, we report the identification and analysis of a homologue of *E.coli* RecA protein in *A. thaliana* that is targeted to the mitochondria.

The putative mitochondrial RecA was identified from the Arabidopsis genome database and a complete cDNA clone was obtained from Kazusa DNA Research Institute, Chiba, Japan. The putative mt-RecA cDNA encodes a protein with high sequence similarity to chloroplast-RecA and several bacterial RecA proteins. Phylogenetic analysis of 17 RecA homologues showed that the mitochondrial RecA was most similar to chloroplast RecA. Interestingly, the sequence was more similar to RecA proteins from the alpha subdivision of the phylum proteobacteria than to *E.coli* (gamma subdivision). The predicted transit peptide was confirmed experimentally by targeting a recombinant transit peptide-GFP(green flourescent protein) fusion protein to mitochondria *in vitro*. The over-expressed mitochondrial RecA protein cross-reacted with anti-chloroplast RecA polyclonal antibodies indicating the conservation of epitopes between the two proteins. Analysis of gene expression along with experiments to determine conservation of the gene in other plant species is in progress. The His6-tagged mitochondrial RecA will be over-expressed in *E.coli*, and the purified protein will be used to characterize strand-invasion activity, substrate specificity and ATP-dependence.

Research supported by the COSAM Dean's Research Initiative (Auburn University), the Alabama Agricultural Experiment Station and a Mentoring Award (Brigham Young University).

312 Identification and charaterization of a novel gene family encoding putative transcriptional regulators in Arabidopsis

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We have identified nine members of a *GRF1-like* (*GRL*) gene family in Arabidopsis genome. All deduced proteins of the GRL family show the same characteristic features of transcription regulators and two conserved regions, the QLQ and WRC domains, as does Os-GRF1 (van der Knaap et al., 2000; Plant Physiol 122;695-704). The C-terminal regions of the GRL proteins are divergent with very limited similarity to the corresponding region of Os-GRF1 as well as to each other. By using GAL4-based yeast system, we demonstrate that At-GRL1 shows transactivation activities for the reporter genes such as LacZ and HIS3, indicating that the GRL gene family encodes putative transcriptional activators. RNA gel blot analysis shows that all the *GRL*s except *GRL9* are highly expressed in the shoot tips and flowers and, with exception of *GRL7* and *GRL8*, in roots as well. To investigate the expression pattern of *GRL1* through *GRL3* in detail, Arabidopsis was transformed with promoter::GUS fusion constructs of each of these genes. At the seedling stage, the transformants showed strong staining in the root meristem, pericycle, or elongation zones. In adult plants, carpels were stained in all three of the transformants; both stems and pedicels were stained in GRL1::GUS and GRL3::GUS, but not in GRL2::GUS transformants. These results indicate that *GRL1* through *GRL3* may play a role in both carpel development and in elongating tissues such as stems, pedicels, and roots. To determine the function of GRL proteins, we have identified T-DNA insertional mutants for *GRL1* through *GRL3*. Sequencing showed that the *grl1-5* has T-DNA insertion in the last exon of *GRL1*, *grl2-93* in the second exon of *GRL2*, and *grl3-24* in last intron of *GRL3*. RNA gel blot analysis showed that *grl1-5* and *grl2-93* mutants lack any detectable transcripts for either gene. We did not observe a phenotype for *grl1-5* and *grl2-93* under different growing conditions and at different developmental stages. We have constructed a *grl1*/*grl2* double mutant and will assess phenotypical changes in the double mutant.

313 The Role of DNA Methylation in Nucleolar Dominance

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Plants that form interspecific hybrids, either naturally or synthetically, may silence parental ribosomal RNA gene arrays and fail to form nucleoli in a phenomenon called nucleolar dominance. We have shown that in the allotetraploid *Arabidopsis suecica* , which is derived from *A. thaliana* and *A. arenosa* , treating with the DNA methylation inhibitor aza-dC and the histone deacetylation inhibitor TSA activates silenced *A. thaliana* genes that have been subjected to dominance. The results of this experiment implicate chromatin effects in nucleolar dominance.

We have begun to investigate the role of DNA methylation in nucleolar dominance. Initial experiments show that both active and silenced rDNA loci are relatively hypermethylated in the intergenic spacer and promoter region; furthermore, particalar accessions of naturally derived *A. suecica* plants show differing degrees of methylation in this region. These naturally occurring strains of *A. suecica* exhibit different degrees of dominance, but the degree of silencing does not correlate with methylation status. This apparent lack of direct correlation between DNA methylation status and degree of silencing lead one to believe that methylation may be acting at some other locus in nucleolar dominance; however, a genetic approach is required to definitively identify molecular components of the methylation machinery involved in establishing nucleolar dominance. Using reverse genetics, we have begun to investigate the role of gene products that may be involved in nucleolar dominance. Recently, we have investigated the role of DDM1 in nucleolar dominance by knocking out the gene in transgenic *A. suecica* using a dsRNA dominant negative strategy. Initial experiments indicate that these experiments knock out expression of both the *A. thaliana* and *A. arenosa DDM1* gene copies. Further experiments will provide genetic and molecular evidence for the role of DNA methylation in nucleolar dominance.

314 Transgene silencing is triggered by transcript level rather than DNA sequence homology or site of T-DNA integration in the *Arabidopsis thaliana* genome

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A predictable and stable transgene expression is a prerequisite for the broad use of transgenic plants. But in transformation experiments variability of transgene expression and even silencing of the transgene is frequently observed. Gene silencing also seems to play a general role in gene regulation.

For a comprehensive study of transgene expression in *A. thaliana*, we established single copy T-DNA lines harbouring reporter genes of distinct kind and numbers in many different genomic positions. We investigated in which way the copy number of a transgene, its position or the presence of other reporter genes influenced its expression. Under the control of the 35s promoter, we used the chimaeric ß-glucuronidase gene, the streptomycin-phosphotransferase gene and the gene for the green fluorescent protein as reporter genes.

Below a certain number of identical transgenes we observed a positive correlation between copy number and reporter gene expression. The expression is high, stable over all generations analysed and comparable between independent lines carrying the same transgene copy number. Most importantly, characterisation of more than 70 independent single copy T-DNA transformants revealed no case of silencing due to genome position.

As soon as a certain number of a particular transgene is reached silencing is triggered. This number is different for distinct transgenes. In contrast, promoterless copies of a transgene do not induce silencing.

Different reporter genes in the same transformant are controlled independently, as long as the transgenes are expressed. But if one transgene is silenced, due to copy number, other reporter genes are silenced as well, regardless of copy number. Probably because the different transgenes share sequence homology in the 5’ and 3’-non-translated regions.

According to these results, triggering of transgene silencing is dependent on transcript level, rather than DNA-sequence homology or the site of T-DNA integration within the *A. thaliana* genome. The presence of 25nt RNAs and resetting at meiosis indicate a post transcriptional mechanism of gene silencing.

315 The development of a screen for Arabidopsis mutants with altered *TCH4* expression profiles

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The *TCH4* gene in Arabidopsis is a mechanosensitive gene that responds to multiple stimuli besides touch, including heat, cold, darkness, IAA, and BR. Evidence suggests that all of these stimuli may be perceived through their mechanical properties. Little is known about the upstream signaling events that influence *TCH4* expression. We have developed a mutant screen with the goal of illuminating the molecular mechanisms which lead to *TCH4* expression. The screen searches for mutants with an altered level of expression of a firefly luciferase reporter gene controlled by the *TCH4* regulatory region known to be responsive to all the known inducing stimuli. The conditions and procedures for the screen have been established and at least one individual with a heritable phenotype has been isolated, proving the viability of the screen. Isolation of mutants with altered expression of *TCH4* will allow insight into the process of mechanosensory signal transduction. These mutants may also help determine the physiological role of the *TCH4* product, a xyloglucan endotransglycosylase.

This work has been supported by DOE (DE-FG03-99ER 20331).

316 Restricted chromosomal silencing in nucleolar dominance

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Washington University

Failure of one parent's chromosomes to form nucleoli in an interspecific hybrid is an epigenetic phenomenon known as nucleolar dominance. Selective chromosomal silencing is involved and operates, at a minimum, on a scale of several million basepairs, the size of a nucleolus organizer region (NOR). The full extent to which NOR-bearing chromosomes are inactivated in hybrids has not been investigated. The recently completed genome sequence of Arabidopsis thaliana allowed a determination of the extent of silencing in Arabidopsis suecica, the allotetraploid hybrid of A. thaliana and A. arenosa. Both NORs of A. thaliana, spanning ~8 Mbp in total , are silenced in A. suecica whereas the NORs inherited from A. arenosa are active. A. thaliana NOR4 abuts the telomere on chromosome 4, thus there are no genes distal to the NOR. We show that the three nearest protein-coding genes flanking NOR4 on its centromere-proximal side remain active in the hybrid despite the silencing of the distal ~4 Mbp. These data reveal that silencing is restricted to the NOR and that repressed and active chromosomal regions in plant genomes can co-exist within several kilobases.

317 Somatic and germinal excision activities of the Arabidopsis transposon Tag1 are determined by distinct regulatory sequences within Tag1

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Various sequence elements of Tag1, the endogenous transposon of Arabidopsis, were investigated to determine how excision and expression are regulated. The 5' intron of the major 2.3 kb Tag1 transcript was found to be critical for the accumulation of Tag1 transcripts and for high rates of somatic excision. This was true for the autonomous element itself and for a two component system using a CaMV 35S expression vector to produce Tag1 transposase and a GUS::dTag1 marker to score for excision. The 3' introns of Tag1, although not needed for high transposase expression in primary transgenic plants, were important for maintaining high levels of somatic excision and accumulation of the major but not minor Tag1 transcripts in subsequent generations. With both 5' and 3' introns present, exchanging the 5' promoter region of Tag1 with the 35S promoter did not significantly affect the timing of Tag1 excision, but it did disrupt germinal excision. Removal of the 5' intron did not abolish germinal excision activity, however. These results indicate that somatic and germinal excision of Tag1 are differentially controlled with the 5' promoter region being critical for germinal excision activity and the 5' intron being important for somatic excision.

318 Centromere conformation and DNA methylation in Arabidopsis

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

319 Expression of a methylated gene family in Arabidopsis

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The Arabidopsis phosphoribosylanthranilate isomerase (*PAI*) genes encode the enzyme that catalyzes the third step in the tryptophan biosynthetic pathway. In the Arabidopsis strain WS, there are four *PAI* genes that are highly identical to one another and heavily cytosine methylated over their identical regions. *PAI* expression analysis has shown that only one gene, *PAI1*, is expressed. Unlike the other WS PAI genes, *PAI1* transcripts can initiate at a novel promoter found approximately 500 bp upstream of the *PAI* methylation boundary. This observation suggests that *PAI1* escapes silencing because the novel promoter is not impeded by methylation further downstream. We are using both RNA analysis and transgenic approaches to test this hypothesis. In particular, we have found that transgene constructs designed to methylate and silence the novel upstream promoter do indeed block *PAI1* expression. This result indicates that in plants, methylation must be near the start of transcription to impair gene expression, implying that methylation inhibits transcription initiation steps, rather than transcript elongation steps.

320 MOLECULAR STUDIES OF SINGLE-COPY SILENCING LINES

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Transgene silencing has been observed in many transgenic plants. Silencing of transgenes may occur by two different mechanisms i.e. transcriptional transgene silencing (TGS), where the transgenic promoters are inactivated or by post-transcriptional transgene silencing (PTGS) where a failure of accumulation of transgenic m-RNA is observed. Many factors have been shown to have an effect on gene silencing including DNA methylation, chromatin structure and positional effects. In addition to these factors, mechanisms such as production of aberrant RNAs and ectopic DNA-RNA interactions can also explain reduced expression levels of the transgene. Although some authors have described silencing of single-copy transgenes, transgene silencing is more pronounced in plants containing multiple copies of the transgene. In our lab, 10 Arabidopsis single-copy transgenic lines displaying transgene (*nptII*) silencing were identified. At this moment we cannot say whether the silencing of the transgene in these plants represents TGS or PTGS. We believe that the genomic position of the transgene in these lines is important. Several mechanisms have been proposed for silencing of single-copy lines, including involvement of repetitive sequences, diverging GC content between the transgene and the surrounding genomic DNA and antisensing by endogenous genes. In our lab, we are studying the genomic DNA flanking the transgenes by the Inverse PCR or by Genome Walking strategy. After cloning the flanking regions we have been able to identify the genomic context of these transgenes and a sequence analysis of these regions will be presented.

321 Cis-acting elements acting to create circadian phase in Arabidopsis

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The circadian clock is an endogenous, molecular timing mechanism that enables organisms to synchronize their biology with the diurnally changing environment. A key outcome of timekeeping is the ability to partition, or phase, distinct biological activities to specific times over the solar day. We have taken a bipartite approach to determine how circadian phase is established at the level of transcription. First, by promoter resection we have isolated a cis-acting circadian clock response element (CCRE) that confers evening-specific transcription. We have defined specific bases within the CCRE, through mutational analysis, that are necessary for circadian regulated expression. Second, we have identified genes transcribed at various circadian phases by screening enhancer trap Arabidopsis lines in which a construct containing a minimal promoter fused to the luciferase coding region was randomly integrated by T-DNA transformation. This allowed the identification of novel clock regulated genes with CCREs in their promoter regions. Both of our approaches reveal a 10 base pair CCRE, AAAAAt/aATCT, which is found in the promoter of genes that are transcribed at circadian phases spanning the solar day. Analysis of circadian microarray experiments confirms that most genes from multiple circadian phases contain the 10 base pair CCRE in their promoters. Since this CCRE is found in promoters transcribed at different circadian phases, we have addressed three possibilities as to the phasing of circadian transcription: base pair changes within the CCRE, context around the CCRE and interaction of the CCRE with other cis-acting elements. This work was supported by grants from the National Science Foundation (MCB9723482 and MCB0091008).

322 Molecular Genetic Analysis of Arabidopsis ETHYLENE INSENTITIVE6

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The plant hormone ethylene regulates a variety of developmental and stress responses, including the classical triple response to ethylene. Exploiting the triple response assay to identify mutants defective in this response ETHYLENE INSENSITIVE6 was previously identified. This mutant was previously shown to suppress the activity of CTR1 and therefore acts downstream of CTR1 in the ethylene pathway. Further characterization of the L(er) allele of this mutant revealed that it contained a second recessive mutation, which dramatically enhanced the *ein6* mutant phenotype. The first mutation was characterized as having an ethylene insensitive root phenotype (EIN6). The second mutation, ENHANCER OF ETHYLENE INSENSITIVITY (EEN), has no phenotype on its own, but changes the *ein6* phenotype from only ethylene insensitivity in the root to a complete lack of the triple response. A search for additional alleles yielded two Col-O T-DNA insertional alleles. Examination of these alleles, located in the promotor and first intron respectively, show that they do not demonstrate the *ein6* phenotype. EIN6 was mapped to the bottom of chromosome 3. Sequence analysis revealed a 7 bp deletion, which causes a frame shift and introduces a stop codon. EIN6 may encode a DNA binding protein, consistent with its position in the pathway. We are currently examining the effects of *een* on the Col-O alleles of EIN6, along with further characterization of *ein6* and *een*.

323 Genetic and molecular analyses of flavonoid metabolism in seed

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It is known that flavonoids which accumulate in the seed coat are determinant for different aspects of the seed quality (*e.g.*, agronomic and industrial properties). This fact prompted us to investigate flavonoid metabolism in the seeds with the aid of genetic and molecular approaches. To this end, we have been studying arabidopsis mutants specifically affected in the seed coat pigmentation. To date, at least 20 loci involved in flavonoid metabolism have been identified in arabidopsis, which were mostly named *TRANSPARENT TESTA* (*TT*). Several of them have been shown to encode enzymes involved in the structure of flavonoid biosynthesis, but data about the regulation of their expression are rather scarce. In our laboratory, the screening of the Versailles T-DNA-mutagenized Arabidopsis collection led to the identification of 22 independent insertional lines showing a modification of the seed coat pigmentation, of which genetic and molecular studies are presented. Two of these mutants represent novel *TT* loci. Besides, T-DNA tagged alleles were identified for four *TT* loci, namely *TT2*, *TT8*, *TT15*, and *TT16*. Molecular cloning of the corresponding genes revealed that they encode a R2R3 Myb domain protein, a bHLH domain protein, a putative glucosyltransferase, and a third transcription factor, respectively. We particularly focussed on the functional analyses of the *TT2* and *TT8* genes. Expression analyses demonstrated that *TT8* transcripts are present in developing siliques and in young seedlings, where flavonoids accumulate. Conversely, *TT2* mRNA was specifically expressed in the endothelium of immature seeds. Additional experiments demonstrated that both TT2 and TT8 modulate the expression of at least two late flavonoid biosynthetic genes, namely *DFR* and *BAN*, in arabidopsis siliques, supporting a major role of the TT2 and TT8 proteins in the flavonoid regulatory network. Interestingly, we showed that the *TTG1* gene is also required for usual expression of *DFR* and *BAN* genes in Arabidopsis siliques. Thus, our results demonstrate that TT2, TT8, and TTG1 act in concert to regulate flavonoid metabolism in the arabidopsis seed coat.

Nesi N. et al., Plant Cell (2000), 12, 1863-1878.

324 Characterization of Arabidopsis S15 Ribosomal Protein Gene Family Expression by Relative RT-PCR.

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The eukaryotic cytoplasmic ribosome, essential for protein synthesis, is comprised of 4 rRNA molecules and approximately 80 ribosomal proteins (r-proteins). Studies in yeast and other organisms demonstrated that these ribosomal components are produced stoichiometrically, indicating coordinate gene regulation. The r-proteins are often encoded by small multi-gene families; in animals, the families are comprised of both a transcribed intron-containing gene and processed pseudogenes. In contrast, the r-protein gene families identified in plants contain multiple intron-containing genes encoding proteins of both identical and similar amino acid sequences. Evidence from the few plant r-protein gene families investigated indicates these multiple genes are expressed, possibly to provide additional quantities of r-proteins in actively growing cells or to provide tissue-specific r-protein variants. We are using the *Arabidopsis* S15 gene family to investigate the temporal and spatial expression of these genes and their regulation during plant growth and development. The S15 family contains five intron-containing genes (*RPS15A - E*), two of which appear as cDNAs or ESTs in the sequence databases. The five genes encode proteins of 149-152 amino acids with 84% or greater amino acid identity. RNA was isolated from *Arabidopsis* plants at 24 hour intervals for the first 16 days post germination and from mature stems, leaves and flowers and analyzed for expression by RT-PCR with gene specific primers from each 3[prime] UTR. Relative comparisons were made to 18S rRNA levels as an internal standard. Four of the genes (*RPS15A-D*) were expressed throughout the time course, with S15 mRNA levels peaking on days corresponding to the development of new leaves and the start of bolting. *RPS15A* and *RPS15B* mRNA levels peaked on different days, indicating these two genes may be differentially expressed. *RPS15A* mRNA was found at higher levels in the leaves and developing flowers than *RPS15B*, but there was no significant difference in mRNA levels within the stems. No *RPS15*E RNA was detected in any tissue or at any time examined.

325 Cloning and Characterization of Sugar-insensitive Mutants

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Sugars are known to be important plant metabolites, but little is known about the role of sugars as signaling molecules or the molecular mechanisms by which sugars exert their effects. To facilitate the unraveling of sugar signaling pathways, we have isolated a number of mutants that are insensitive to the inhibitory effects of high levels of exogenous sugar on true leaf development. Wild-type plants will not form expanded cotyledons or true leaves on high sucrose or glucose media. Mutants were isolated by screening T-DNA tagged populations on 0.3-0.34M sucrose media and selecting plants that form true leaves.

The sugar-insensitive 3 (sis3) mutant was isolated from a T-DNA tagged line. The *sis3* mutant is not defective in all sugar responses. Unlike other known sugar response mutants, *sis3* has a wild-type response to gibberellin, abscisic acid, ethylene, auxin, cytokinin, and methyl-jasmonate This suggests that the *sis3* mutation may play a relatively direct role in sugar signaling. We are in the process of mapping *sis3* as a possible first step towards isolating the *SIS3* gene using a map-based approach. So far, *sis3* has been mapped to a region of chromosome 1 near the nga 280 marker. The T-DNA border sequences appear to be re-arranged or truncated as attempts to clone the DNA sequences flanking the insert through TAIL-PCR have failed.

A number of additional sugar-insensitive mutants have been isolated from T-DNA tagged lines in which each T-DNA contains a cDNA driven by a 35S promoter. Five different types of mutants are possible: (1) disruption of a gene by the T-DNA insert, (2) random mutations unlinked to the T-DNA insert, (3) over-expression of the cDNA, (4) anti-sense expression of the cDNA, and (5) co-suppression of the cDNA. We are currently in the process of sequencing the cDNA inserts and the DNA flanking the inserts for each of our mutants. We have begun conducting the genetic analyses to determine whether the T-DNA is linked to the mutation and have begun characterization of the mutants. Supported by Department of Energy Biosciences Program Grant #DE-FGO3-00ER15061

326 DNA damage induced cell cycle checkpoints in Arabidopsis

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We are interested in the basis for radiation-induced arrest of Arabidopsis growth. When seeds are irradiated they germinate at a normal rate and form small plants, but these plants stay arrested as seedlings for several weeks. Because early plant growth is dominated by cell expansion rather than cell division we believe the meristematic cells are arrested in the cell division cycle. Our investigations as to the basis of this arrest demonstrate that the meristematic cells are arrested in the G2 phase of the cell cycle. We conducted a screen for mutants that did not arrest following gamma-radiation. Five plants falling into two complementation groups were identified that continued to form new leaves following gamma-radiation. These mutants have been termed *sog* (suppressor of gamma). These mutants do not arrest in the G2 phase following gamma radiation and display high levels of chromosomal instability.

327 DAG: A gene family involved in plastid development

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The dag mutant in A.majus is defective in leaf pigmentation due to the complete arrest of chloroplast development in the mutant areas. In mutant sectors, the palisade cells fail to develop and expand properly. The development of the etioplasts in dark is also affected. DAG is required for the expression of plastid encoded genes, very early during plastid development. DAG belongs to a gene family unique to plants, whose products show a transit peptide and a highly conserved N-terminal region. There are ten DAG genes in Arabidopsis. We have cloned cDNAs for 8 of these family members, including AtDAG1, the orthologue to the A.majus DAG gene. The dal1 mutant of Arabidopsis has a T-DNA insertion which blocks the expression of AtDAG3. In these plants, chloroplasts fail to develop, although a low level of chlorophyll may be synthesised. Genes encoding subunits of the plastid encoded polymerase (PEP) are expressed, unlike in the dag mutant of A.majus. We have isolated insertion mutants in AtDAG1, AtDAG2 (from Arabidopsis KO facility at the University of Wisconsin) and AtDAG5 (from the collection at the Univ. of Gent), but we have been unable to identify homozygous lines. Gaps observed in the siliques of the heterozygous plants suggest that the mutations are seedling lethal. We are crossing heterozygous plants with plants carrying a dominant visible marker linked to each one of the three genes. We should expect all plants in F2 to carry the dominant marker. We are also proceeding with the rescue of the homozygous mutants. We will transform the heterozygous plants with a construct containing each gene cloned after a double 35S promoter. Strong expressions should compliment the mutation, but the double 35S promoter also gives rise to weak expressions, which will, hopefully, give weak, viable phenotypes. The effect of the mutations on plastome expression will also be investigated. At the moment we are amplifying by PCR all the 87 proteins, 4 rRNAs and 37 tRNAs from the plastid genome in Arabidopsis. These PCR products will be spotted onto filters, providing a tool to assess the effect of DAG genes on plastome transcription. These and other experiments will shed light on the DAG family function in plastid and plant development.

328 CONSEQUENCE OF TELOMERE DEPLETION IN *ARABIDOPSIS*

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We are studying telomere function and maintenance in *Arabidopsis thaliana*. We have identified an *Arabidopsis* line containing a T-DNA disruption in the gene encoding the telomerase catalytic subunit (*AtTERT*). The telomerase deficiency leads to telomere shortening and to a dramatic decrease in TRF heterogeneity, wherein the “fuzzy” distribution of TRFs in wt plants is replaced by a series of discrete bands in mutants. Experiments with subtelomeric probes specific for individual chromosome ends demonstrated that each band represents a unique chromosome arm. Although the overall rate of telomere shortening is 200-500 bp per plant generation, dramatic increases and decreases in some TRFs were observed, consistent with stochastic recombination and/or gene conversion events. Unlike the alternative lengthening of telomeres described for telomerase-negative yeast and cultured mammalian cells, these events occur in earlier generations (G1 – G3) of mutants before any cytogenetic abnormalities were observed. Telomere dysfunction results in chromosome fusions, cell cycle arrest, senescence and apoptosis in telomerase-deficient mice and yeast. The first chromosome fusions were detected as anaphase bridges in G5 *Arabidopsis* mutants and their frequency increased in subsequent generations. The first developmental defects appeared in G6 and correlated with the onset of chromosome fusions. Growth aberrations were caused by insufficient cell proliferation. Late generation mutants exhibited small asymmetric leaves, altered phyllotaxy and disorganized shoot apical meristems. Phenotypes progressively worsened from mild defects in leaf morphology to developmental arrest and sterility in subsequent generations. Interestingly, programmed cell death does not appear to be associated with telomere dysfunction in *Arabidopsis* as neither DNA fragmentation nor chlorophyll degradation were associated with the most severely affected leaves. In addition, some plants with severe developmental defects had a substantially longer life span than their wt counterparts. These observations suggest that aspects of the primary cellular response to telomere dysfunction are different in plants and animals.

329 How Do Introns Elevate Gene Expression?

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Introns are known to boost gene expression in many organisms including plants, but the means by which they do this are not understood. To explore potential mechanisms, I am analyzing the requirements for introns to enhance mRNA accumulation from a *PAT1:GUS* fusion in transgenic Arabidopsis. Three areas are being investigated: the intron structural components that are needed, the variation between introns in expression-enhancing ability, and the importance of intron position. Derivatives of *PAT1* intron 1 containing a mutant 5' splice site or lacking branchpoint sequences still elevate expression, even though they are not spliced. These findings rule out direct involvement of the U1 or U2 snRNPs (which initiate spliceosome assembly by binding to the 5' splice site and branchpoint, respectively) and the need for splicing per se. Similarly, none of a series of deletions that together span the intron eliminate the ability to stimulate expression. Any intron sequences that are involved must therefore be redundant. The ability of seven other introns to stimulate *PAT1:GUS* expression relative to an intronless fusion ranged from under 2-fold to more than 30-fold, and the degree of enhancement correlated with intron length and U-richness. A possible role for U-rich sequences is supported by the finding that *PAT1* intron 1 was less effective at stimulating mRNA accumulation when it's U-content was reduced. *PAT1* intron 1 also failed to elevate expression when inserted 25 nt downstream of the stop codon, despite being efficiently spliced. This suggests that introns affect expression during, rather than after, transcription. One possible mechanism is that factors that bind to a recently transcribed intron could mediate a change in the carboxy-terminal domain of RNA polymerase II. This part of the polymerase is involved in regulating the rate of transcription and is known to bind several enzymes involved in mRNA maturation, including splicing factors. An interaction with intron-bound factors could render the polymerase more processive and therefore more likely to extend transcription to the end of the gene, where proper 3' end processing stabilizes the transcript. The intron components required for this modification must be distinct from (but may overlap with) those that mediate intron recognition for splicing, and may involve redundant elements such as U-rich sequences.

330 Comparison between biennialism in *Hyoscyamus niger* and winter annualism in *Arabidopsis thaliana*

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There are genetic similarities between the biennial growth habit of *Hyoscyamus niger* (*H. niger*) and winter annual ecotypes of *Arabidopsis thaliana*. Biennialism is dominant over annualism, but F1 hybrids are converted to very late-flowering winter annuals. This resembles the genetic interaction between *FRIGIDA* (*FRI*) and certain alleles of *FLOWERING LOCUS C* (*FLC*) in *Arabidopsis*. We show here that another similarity includes the response of *H. niger* to demethylating agents. We also present results towards determining whether *FLC* homologs or *FLC*-related genes are involved in biennialism, and summarize progress towards isolating MADS-box sequences from biennial *H. niger*. Results from our initial characterization of expression profiles of four groups of MADS-box genes suggest that B-class floral homeotic gene homologs are differentially expressed in flowers of annual and vernalized, biennial *H. niger*.

331 Expression of an SMC2 gene during mitosis and meiosis in Arabidopsis: Antisense inhibition perturbs morphogenesis at the shoot apex

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The SMC (Structural Maintenance of Chromosomes) proteins can be divided into five distinct subfamilies, whose members play vital roles in chromosome condensation, cohesion, recombination, and dosage compensation. Members of the SMC2 subfamily are involved in coordinating mitotic chromosome condensation. We have cloned the first plant cognate of the SMC2 family (AtSMC2-1) from Arabidopsis. The AtSMC2-1 cDNA effectively rescued the yeast smc2-δ6 temperature sensitive mutant, suggesting that AtSMC2-1 may regulate chromosome condensation in Arabidopsis. Sequence database analyses indicated that there is a second gene, AtSMC2-2, which is 83% identical to AtSMC2-1. RT-PCR followed by CAPS analysis provided evidence for differential expression of the two genes, with AtSMC2-1 being the more highly expressed. Transgenic plants harboring an AtSMC2-1::GUS transgene exhibited GUS staining in spatial patterns reflecting the mitotic activity of each tissue. Although the role of SMC2 proteins in mitotic chromosome condensation has been studied extensively in yeast and animal systems, no evidence for its involvement in meiotic chromosome condensation has yet been reported from any organism. Our in-situ hybridization results demonstrated that AtSMC2-1 mRNA is present in meiotic cells, suggesting a role for these proteins in meiotic chromosome condensation. Lastly, in order to examine the consequences of reduced levels of the AtSMC2-1 protein, we generated transgenic Arabidopsis plants constitutively expressing an antisense fragment of the AtSMC2-1 gene. These antisense transgenic plants exhibited extremely slow root and shoot growth, enlarged and dysfunctional shoot apical meristem, fasciated stems, and altered leaf arrangement. Characterization of these defects at the cytological and molecular level will be presented at the meeting.

332 Genetic Modifiers of *Ac* Transposition in *Arabidopsis*

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The *Arabidopsis increased Ac excision 1*(*iae1*) mutant was generated by gamma-ray mutagenesis of a line carrying a single copy of the *Activator* (*Ac*) transposon. The mutation causes a 550-fold increase in the number of *Ac* excision events per cotyledon. It is unlinked to the *Ac* element and inherited in a Mendelian fashion. Previously, *iae1* was mapped to the short arm of chromosome 2 using a mapping population of 42 plants (Jarvis *et al*., 1997, *Plant J*. 11: 907-919). Using a larger population (860 plants) and closely linked markers, fine-mapping of *iae1* has now been completed. The gene responsible for the *iae1* phenotype has been tentatively identified. During the mapping of *iae1*, a number of additional, unlinked loci that have modifier effects on *Ac* mobility and/or the *iae1* phenotype were identified. Finally, new screens of EMS and X-ray mutagenized populations for *iae*-like mutants are being conducted. To date, approximately 50 new mutants have been identified. Progress on these various projects will be reported.

333 The C-Terminal 98 Amino Acids of Arabidopsis CBF1 Functions as a Transcriptional Activation Domain and Causes Severe Growth Retardation when Overexpressed in Transgenic Arabidopsis as a Fusion with the DNA Binding Domain of the Yeast GAL4 Protein.

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The CBF proteins are transcriptional activators that play an integral role in plant cold acclimation, the phenomenon whereby certain plants, including Arabidopsis, increase in freezing tolerance in response to low nonfreezing temperatures. The CBFs bind to the CRT/DRE cold- and drought-responsive DNA regulatory element and activate expression of *COR* (cold-regulated) and other genes that have the CRT/DRE element in their promoters. Constitutive high-level overexpression of the CBFs in transgenic Arabidopsis results in increased freezing tolerance (3-5). However a negative consequence often associated with CBF overexpression is severe growth retardation (4,5), a phenotype thought to result from constitutive overexpression of the CBF-targeted genes (4). The results presented here, however, raise an additional possibility. We define the CBF1 DNA-binding, $CBF1_{DBD}$, and trans-activating, $CBF1_{AD}$, domains and show that overexpression of a fusion protein consisting of $CBF1_{AD}$ joined to the DNA binding domain of the yeast GAL4 protein, $GAL4_{DBD}$, in transgenic Arabidopsis results in severe growth retardation. The CBF-targeted *COR* genes are not activated in these plants indicating that their expression was not the cause of the growth retardation phenotype. We propose that overexpression of the $CBF1_{AD}$ results in growth retardation due to "squelching," a phenomenon similar to that described for yeast and mammalian cells in which excess activator concentrations titrate target proteins causing slow growth (6,7). This has important implications in designing strategies to use transcription factors to modify plant traits. This research was supported in part by the USDA (NRICGP), NSF and Michigan AES to MFT and by the OSU/OARDC to EJS.

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334 *COP10*, a negative regulator of photomorphogenesis, encodes a novel ubiquitin-conjugating enzyme variant

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Light as a signal plays a critical role in regulating plant growth and development. Under the light, *Arabidopsis* seedlings have short hypocotyls, no apical hooks, expanded cotyledons with developed chloroplasts, differentiated cell types and show a dramatic induction of the transcription of many genes. In *Arabidopsis*, genetic analysis has identified mutations in *COP/DET/FUS* loci exhibiting a pleiotropic constitutive photomorphogenic phenotype, suggesting that these genes play an essential regulatory role in the repression of photomorphogenic developments. Our previous research revealed that the *cop/det/fus* mutants can be categorised into three classes based on their effects on the presence of the COP9 signalosome and monomeric forms of the AJH proteins, which is known as CSN5, one of the component of COP9 signalosome. *cop10* is categorised as class II mutants, which has both CSN5 monomer and COP9 signalosome, while class I mutants (*cop1* and *det1*) have COP9 signalosome but no detectable CSN5 monomer, and class III mutants (*cop8, cop9, cop11*) lacking COP9 signalosome but no effect on CSN5 monomer. Thus COP10 defines a subclass of its own, whose role in repressing photomorphogenesis does not involve structural alteration of the COP9 signalosome. Here, we report the identification of a *CONSTITUTIVE PHOTOMORPHOGENIC 10 (COP10)*, encoding a novel type of ubiquitin-conjugating enzyme E2 variant (UEV), lacking a recognizable catalytic center of ubiquitin-conjugating enzymes (E2).

335 PCR-assisted *in vitro* binding site selection for AGL15, an embryo MADS-domain factor

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MADS-domain proteins are a family of DNA binding transcription regulators. AGL15 is currently the only reported MADS-domain protein preferentially accumulated during plant embryo development. All MADS factors share a highly conserved MADS-domain responsible for DNA binding and protein dimerization. Previous studies have shown that MADS-domain proteins recognize the so-called CArG motif, with a consensus nucleotide sequence of $CC(A/T)_6GG$. However, different MADS-domain proteins have similar, yet distinctive binding sequences. This difference in binding site preference may be critical for differential gene regulation. To determine the consensus sequence AGL15 preferentially binds *in vitro*, a PCR-assisted binding site selection assay was performed. T7-tagged AGL15 protein was expressed in *E.coli* and immunopurified. A pool of oligonucleotides with a core of 26 random bases was used with AGL15 for electrophoretic mobility shift assay (EMSA). Shifted sequences were amplified by PCR and subjected to further rounds of EMSA. After 3 to 5 rounds of selection, shifted oligos were sequenced. Multiple sequence alignment revealed AGL15 prefers a CArG motif with longer AT-rich core, apparently different from the known binding sites of other plant MADS-domain proteins. The biological significance of this difference in binding preference is currently under investigation.

Supported by the University of Kentucky, Department of Agronomy, and by the National Science Foundation (IBN-9984274).

336 Characterization of two SET-domain encoding Arabidopsis genes with similarity to Drosophila SUPRESSOR OF VARIEGATION (3-9)

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Gene expression in eukaryotes depends on both intrinsic regulatory mechanisms, including enhancer-promoter interactions, and chromosomal context, including chromatin structure. An understanding of the mechanisms governing modulation of chromatin structure is emerging from the identification of genes encoding proteins that form chromatin complexes. In *Drosophila*, the chromosomal proteins SUPPRESSOR OF VARIEGATION 3-9 [SU(VAR)3-9], ENHANCER OF ZESTE [E(Z)] and TRITHORAX [TRX], which are involved in epigenetic control of gene expression and Position Effect Variegation (PEV), share a common 130 amino acid motif, the SET-domain. *CURLY LEAF* and *MEDEA*, encoding proteins with high similarity to E(Z), were the first SET-domain genes to be identified in plants.

We have cloned the transcripts of two Arabidopsis genes encoding proteins with high similarity to the SU(VAR)3-9 protein using RT-PCR and RACE. To characterize these genes we have studied their expression patter in various tissues and at different developmental stages. To investigate their function in the plant, Arabidopsis has been transformed with RNA interference constructs aimed at knocking out gene expression

337 Histone deacetylation and epigenetic regulation in Arabidopsis

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Histone acetylation and deacetylation play essential roles in eukaryotic gene regulation. Reversible modifications of core histones are catalyzed by two intrinsic enzymes, histone acetyltransferase (HAT) and histone deacetylase (HD or HDAC). In general, histone deacetylation is related to transcriptional gene silencing, while acetylation correlates with gene activation. We produced transgenic plants expressing antisense Arabidopsis histone deacetylase (AtHD1) gene, a homolog of the HD1 in human and RPD3 global transcriptional regulator in yeast. Expression of antisense AtHD1 caused dramatic reduction in endogenous AtHD1 transcription, resulting in accumulation of acetylated histones, notably tetra-acetylated H4. Reduction in AtHD1 expression and AtHD1 production and changes in acetylation profiles were associated with various developmental abnormalities, including early senescence, ectopic expression of silenced genes, suppression of apical dominance, homeotic changes, heterochronic shift towards juvenility, flower defects, and male and female sterility. Some of the phenotypes could be attributed to ectopic expression of tissue-specific genes (e.g., SUPERMAN) in vegetative tissues. No changes in genomic DNA methylation were detected in the transgenic plants. These results suggest that AtHD1 is a global regulator, which controls gene expression during development through DNA-sequence independent or epigenetic mechanisms in plants. In addition to DNA methylation, histone modifications may be involved in a general regulatory mechanism responsible for plant plasticity and variation in nature.

338 A two member gene family encodes the L3 ribosomal protein in Arabidopsis thaliana

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Ribosomes are essential organelles composed of ribosomal RNA (rRNA) and ribosomal proteins (rProteins). Ribosomes have been studied in some detail in bacteria, yeast and other organisms, and to a lesser degree in plants. Because stoichometric amounts of each rProtein must be present for efficient assembly and the number of genes involved, rProtein genes have been the subjects of studies in coordinated gene regulation.

In *E. coli* each rProtein is encoded by a single gene, in saccharomyces a single gene or a two-member gene family encodes each protein. In plants a small gene family with two or three members encodes most proteins. The deduced amino acid sequence of each member varies from identical to 74% identity. This has made it difficult to determine if products from all of the gene families are incorporated into ribosomes and if some of the family members have specialized roles.

We have characterized two ribosomal protein genes (Arp1 and Arp2) from Arabidopsis that encode a homolog of the *E. coli* L3 protein. These two genes are relatively divergent, sharing approximately 75% nucleotide identity and 85% amino acid identity. Arp1 appears to be constitutively transcribed at high levels in all tissues. Its mRNA is polyadenylated and can be isolated from the polyribosome fraction. The protein can be isolated from shoots and it is found on polyribosomes in high abundance.

Arp2, appears to be transcribed in all tissues, it is more abundant in root than shoot. Its mRNA has several unusual features. First it has a very short 5' UTR. Despite its short length it does have a CT rich region, typical of the 5' UTR of ribosomal protein mRNAs. Second, its mRNA is found in the poly A- fraction after separation on an oligo-dT column. Sequence analysis and RT-PCR using an oligo-dT primer for first strand synthesis has located a polyadenylation site. At this site a short polyA tail appears to be present on at least a majority of the messages. Arp2 mRNA can also be isolated from polyribosomes; Western analysis confirms the presence of Arp2 protein in shoot polyribosomes, but at an apparently lower concentration than Arp1.

339 FHY3 encodes a novel nuclear protein essential for phytochrome A signaling in *Arabidopsis*

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Phytochrome A (phyA) is the primary photoreceptor mediating responses to the far-red light signal (FR). A significant number of components acting either positively (FHY1, FHY3, FAR1, HFR1, FIN219, PAT1, FIN2) or negatively (SPA1 and EID1) in phyA signaling have been identified and some of these genes have been characterized at the molecular level. Our genetic and physiological analyses indicate that phyA signaling in *Arabidopsis*involves multiple branches controlling overlap yet distinctive sets of photomorphogenic responses (hypocotyl elongation, cotyledon opening and greening, anthocyanin accumulation, gravitropic response, light-regulated gene expression etc.). These branches converge at or upstream of the photomorphogenic repressor, COP1 and regulate its subcellular localization, in turn they control the accumulation of a positive regulator of photomorphogeneis, the bZIP transcription factor HY5. FHY3 represents one of the branches and its loss-of-function mutant displays severely reduced sensitivity to far-red light. *fhy3*mutants possess normal level and photoreactive phyA protein. Therefore, FHY3 likely represents an authentic and essential signal transducer for phyA signaling. Positional cloning of FHY3 indicates that it encodes a novel nuclear protein. FHY3 expression is slightly repressed by FRc but significantly induced by white light. In addition, FHY3 expression is impaired in the *fin219*and *spa1*mutant backgrounds and increased in the *far1*mutants, suggesting a complex regulatory network controlling its expression. The FHY3 protein is constitutively localized to the nucleus. Interestingly, overexpression of either the N-terminal or C-terminal fragments of FHY3 causes a dosage-dependent dominant-negative effect on phyA signaling in *Arabidopsis*. Further, FHY3 is able to interact with FAR1 in a yeast two-hybrid assay and an in vivo co-immunoprecipitation assay. Our results suggest that FHY3 and FAR1 play distinctive but interacting roles in phyA signaling, most likely as transcriptional regulators required for phyA induced gene expression and photomorphogenic development.

340 Analysis of Putative Regulatory Targets of AGL15, an Embryo MADS-Domain Factor

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AGL15 (*AGAMOUS-like 15*) is a member of the MADS-box family that is preferentially expressed during embryogenesis. Identifying what genes are regulated by AGL15 is important for understanding the roles of this DNA-binding protein in development. Chromatin immunoprecipitation (CHIP) is an approach to isolate *in vivo* protein-DNA complexes. Several putative target genes of regulation by AGL15 were obtained by using a CHIP method developed in our lab. The expression levels of these genes were compared among various staged tissues in wildtype and transgenic Arabidopsis that overexpress different forms of *AGL15*. The results indicated that some of the genes are down-regulated and others are up-regulated in response to AGL15. Furthermore, potential binding sites for MADS-domain proteins called CArG motifs were found in the regulatory regions of these genes. The specific binding of AGL15 to one of these CArGs has been demonstrated by gel mobility shift assay. More work to confirm that these genes are regulated by AGL15 and to investigate their roles in development is underway.

Supported by the University of Kentucky, Department of Agronomy, and by the National Sciences Foundation (IBN-9984274).

341 Regulation of Ethylene Biosynthesis in *Arabidopsis*

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The plant hormone ethylene is a simple gaseous molecule that governs many aspects of plant growth and development. Mutations in the *ETHYLENE-OVERPRODUCER1 (ETO1)* gene from *Arabidopsis* cause overproduction of ethylene. The recessive nature of the mutations in *ETO1* suggests that it encodes a negative regulator of ethylene biosynthesis. *ETO1* is a member of a novel gene family with BTB/POZ and TPR domains that are highly conserved in the plant kingdom. Mutations in the TPR domain of ETO1 confer overproduction of ethylene, suggesting its essential role. A dominant mutation of another *eto* complementation group, *eto2-1*, was mapped to the *ACS5* gene that encodes a key enzyme of ethylene biosynthesis. This mutation results in an alteration of the last 12 amino acids of ACS5 and confers overproduction of ethylene. Two-hybrid experiments revealed direct interaction of ETO1 with ACS5. Interestingly, the *eto2-1* version of ACS5 can no longer interact with ETO1 in the yeast two-hybrid system. Furthermore, functional assays using a bacterial system and suppression of the *eto1* mutation by a loss-of-function allele of ACS5 reveal that ETO1 and related ETO1-LIKE (EOL) proteins significantly inhibit the activity of ACS5. These results clearly demonstrate the direct inhibition of ACS activity by the ETO1 family and a proposed model is presented.

342 Isolation and Characterization of the y9-287 Meiotic Mutant of Arabidopsis

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Meiosis represents a highly ordered series of events that results in the production of haploid gametes; it plays a central role in the reproduction of essentially all diploid organisms. It lies at the heart of eukaryotic genetic diversity and is most likely responsible for inter-specific breeding barriers. As an experimental system, meiosis provides a valuable system to study many aspects of cellular function, including changes in chromatin conformation, recombination, nuclear and cellular division, and cell cycle control mechanisms.

As part of studies to isolate and characterize genes that are essential for meiosis we have isolated the male sterile *y9-287* mutation from a population of Ds mutagenized plants. Meiosis appears to proceed normally up to mid/late prophase in *y9-287* plants when some microsporocytes begin to exhibit properties of programmed cell death, including shrinkage and condensation of the cytoplasm and abnormal condensation, segregation and degradation of the chromosomes. Microsporocytes arrest at various stages of meiosis with most cells arresting by anaphase I. Analysis of the gene responsible for the *y9-287* mutation identified a PHD domain containing protein. The phenotype of the *y9287* mutation and the presence of nuclear-localization signals and the PHD domain suggest that Y9287 may participate in controlling meiotic gene expression or chromatin remodeling during meiosis. Data will be presented on the isolation and characterization of the *y9-287* mutation and gene.

343 Comparative genome analysis in crucifers

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Genome colinearity has been studied for two closely related diploid species of the Brassicaceae family, *Arabidopsis thaliana* and *Capsella rubella*. Comparative genetic mapping revealed extensive colinear segments for the two species. Detailed analysis of a 200 kbp region in *A. thaliana* and its counterpart in *C. rubella* showed almost complete conservation of gene repertoire, order, spacing and orientation. Only minor differences in microstructure were found, including the insertion of mobile elements, a tandem gene duplication and a single gene deletion/insertion. Alignment of *Arabidopsis* cDNA and EST sequences with genomic DNA sequences of *Arabidopsis* and *C. rubella* showed conservation of exon length and intron positions. Coding sequences predicted from these alignments differed from the annotated *Arabidopsis* gene sequences in a number of cases.

In the paleopolyploid species *Brassica oleracea* at least three partial copies were found corresponding to the 200 kbp *A. thaliana* region. Gene order in *B. oleracea* is similar to the one in *A. thaliana*, however, evidence for translocations, inversions and numerous deletions was obtained. As a result, the triplicated chromosome segments show differences with respect to gene content when compared to each other and to the *Arabidopsis* region. Interestingly, for a number of *Arabidopsis* genes only partial copies were found in *B. oleracea*.

Almost complete microcolinearity was found for the close relatives *A. thaliana* and *C. rubella*, whereas the gene arrangement in the paleopolyploid species *B. oleracea* revealed more extensive differences when compared to that in the other two species. This result can only partially be accounted for by the more recent divergence of the species pair *A. thaliana* and *C. rubella* with respect to *B. oleracea*. Rather, the accelerated rate of change observed in triplicated segments of the *B. oleracea* genome may indicate that duplications/polyploidy foster rapid chromosomal evolution.

344 Consensus and Variation among 180-bp Repeats in Arabidopsis Centromeres

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The recently completed genome sequence of Arabidopsis thaliana has given a particularly insightful view of a plant centromere. Similar to many higher eukaryotic organisms, A. thaliana centromeres contain satellite DNA that is AT-rich and organized tandemly in a head-to-tail fashion, with each monomer being ~180 base pairs long. These 180-bp repeats make up a significant portion of the Arabidopsis genome and are located exclusively within the centromere. Since the centromere is such a unique environment within the genome (a heterochromatic region with low recombination rates), studying the molecular evolution of the 180-bp repeats in different A. thaliana ecotypes and closely related species should give new insights into the evolution of satellite DNA under unique selective pressures. Consensus sequences for the 180-bp repeats were derived for approximately 40 ecotypes of A. thaliana. Alignment of the consensus sequences has revealed that nucleotide substitutions vary within the 180-bp repeat, with some regions being highly conserved and others highly variable.

345 MOLECULAR EVOLUTION OF THE RECQ-LIKE GENE FAMILY IN ARABIDOPSIS THALIANA AND CAPSELLA RUBRELLA

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Members of the RecQ family of DNA helicases are involved in processes linked to DNA replication, DNA recombination and gene silencing. These helicases are conserved throughout all kingdoms except of the archaebacteria. Eubacteria and yeast as well as fission yeast contain only one RecQ or RecQ-like gene. Other fully sequenced eucaryotic genomes possess more RecQ-like genes (*Drosophila melanogaster* (4), *Caenorhabditis elegans* (4), human (5) and *A. thaliana* (7)) which seem to be all functional. In *A. thaliana* two of these 7 genes (AtRecQl 4A and 4B) arose from a recent duplication event whereas the other 5 genes must be products of very old duplication events. Furthermore, *A. thaliana* harbours in comparison to mammals an unusual high number (5) of genes coding for "small" RecQ-like homologues. The only two large RecQ-like homologoues arose by the recent duplication event. Surprisingly the exonuclease domain which is present besides the helicase domain in the werner syndrome gene (WRN) in mammals is coded in Arabidopsis by an independent open reading frame but is able to interact with at least one "small" homlogue (Hartung et al.,2000; NAR Vol 28 No. 21: 4275-4282).

To learn more about molecular evolution of a gene family consisting of ancient and recent paralogs we analyzed the RecQ homologues of the near relative of *A. thaliana*, *Capsella rubrella*. We isolated all 7 RecQ-like genes and the small WRNexo gene from *C. rubrella* as partial (CrRecQl 1 to 5 and WRNexo) or full length sequences (CrRecQl 4A and B). Using the sequence data we could compare the molecular evolution of the duplicated genes and the singletons on the level of nucleotide divergency. The data provided allowed us 1) to calculate mutation rates of singletons and the duplicated genes 2) to fix the duplication event relatively accurate in time.

346 Correlating the Brassica Genetic Map with the Arabidopsis Physical Map

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Cultivated Brassica species are closely related to Arabidopsis, and thus provide an excellent crop model system to test hypotheses of genome evolution and synteny. Here, we report a correlation of the genetic map of Brassica with the physical map of Arabidopsis using a bioinformatics approach. We determined the genomic location within Arabidopsis of over 150 Brassica clones that have previously been used as molecular markers in Brassica linkage maps. A BLAST comparison of the 5' and 3' sequence ends of the Brassica mapping clones with GENBANK accessions was used to determine the position of similar sequences within the Arabidopsis genome. The comparative Arabidopsis and Brassica map supports the hypothesis that highly similar sequences shared between the genera often have conserved genomic positions, although it also provides evidence for genomic rearrangements. Based on hits between less highly conserved sequences, our data also reveal the locations of putative, ancient genome duplications. Finally, we have developed a statistical application to test the significance of collinear regions.

347 *DEFH28*, a novel MADS-box gene from *Antirrhinum majus*, affects floral meristem identity and fruit maturation

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DEFH28 is a novel MADS-box gene from *Antirrhinum majus*. Phylogenetic reconstruction indicates that it belongs to the *SQUA*-subfamily of MADS-box genes. Expression analyses and transgenic plant studies suggest two distinct functions of *DEFH28* effecting early and late flowering processes. Firstly, *DEFH28* is expressed in the inflorescence apical meristem and might control together with *SQUAMOSA (SQUA)* floral meristem identity in *Antirrhinum*. Additionally, *DEFH28* is sufficient to switch inflorescence shoot meristem to a floral fate in transgenic *Arabidopsis* plants. Secondly, *DEFH28* is predominantly expressed in carpel walls, where it seems to exert a function in the regulation of carpel wall differentiation and fruit maturation. Support for this later role comes from overexpression studies of *DEFH28* throughout siliques in transgenic *Arabidopsis* plants where it altered the identity of the replum and valve margin cells towards adapting a valve cell identity. This results in the formation of non-dehiscent siliques. This late aspect of the *DEFH28* function is identical to the *FRUITFULL (FUL)* function in *Arabidopsis* as demonstrated in gain-of-function plants. *FUL*, like *DEFH28*, belongs to the *SQUA*-subfamily of MADS-box genes. *DEFH28* represents the first characterized and most likely, ortholog of *FUL*. Although the overall flower morphology between *Antirrhinum* and *Arabidopsis* plants is highly conserved, their carpels mature into different types of fruits: capsules and siliques, respectively. Therefore, it seems that control of carpel wall differentiation by *DEFH28* and *FUL* involves a conserved molecular mechanism integrated into two very different carpel developmental pathways.

348 KNOX class of homeobox genes potentially have similar function in both sporophytic unicellular and multicellular meristems, but not in gametophytic meristems.

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Members of the class 1 KNOX (knotted-like homeobox) gene family are important regulators of shoot apical meristem development in angiosperms. To determine if they function similarly in non-seed plants, three KNOX genes (two class 1 genes and one class 2 gene) from the fern *Ceratopteris richardii* were characterized. Expression of both class 1 genes was detected in the shoot apical cell, leaf primordium, and marginal part of the leaves , and vascular bundles by in situ hybridization, a pattern that closely resembles that of class 1 KNOX genes in angiosperms with compound leaves. The fern class 2 gene was expressed in all sporophytic tissues examined, which is characteristic of class 2 gene expression in angiosperms. All three *CRKNOX* genes were not detected in gametophyte tissues by RNA gel blot analysis. *Arabidopsis* plants overexpressing the fern class 1 genes resembled plants that overexpress seed plant class 1 KNOX genes in leaf morphology. Ectopic expression of the class 2 gene in *Arabidopsis* did not result in any unusual phenotypes. Taken together with phylogenetic analysis, our results indicate that 1) the class 1 and 2 KNOX genes diverged prior to the divergence of fern and seed plant lineages; 2) the class 1 KNOX genes function similarly in seed plant and fern sporophyte meristem development despite their differences in structure, 3) KNOX gene expression is not required for the development of the fern gametophyte, and 4) the sporophyte and gametophyte meristems of ferns are not regulated by the same molecular mechanisms.

349 Nucleotide changes that caused morphological evolution in diploid and tetraploid wild *Arabidopsis* species

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One of the useful approaches to study evolution and diversity is molecular genetic analysis using closely related species of model organisms. So far, only *Drosophila* species group has been well studied. We collected 25 species that are relatives of *A. thaliana* from Japanese mountains and stock centers, and crossed with *A. thaliana*. Two of them, *Arabidopsis lyrata* and *Arabidopsis halleri*, yielded hybrid F1. Using the hybrids, we conducted "interspecific allelism test".

Ohara line of *A. halleri* and Tottori line of *A. lyrata* subsp. *kamchatica* has no trichomes on the leaves. Interspecific allelism test with trichomeless mutants of *A. thaliana* showed that both lines are defective in *GLABROUS1* gene, which encodes a myb transcription factor and is essential for trichome cell differentiation in the epidermis layer.

To identify the mutation at the nucleotide level, we isolated *GLABROUS1* homologues from Ohara line of *A. halleri*. The line is diploid, and one *GLABROUS1* gene was isolated. DNA sequencing analysis revealed a frameshift mutation owing to AA to C base change at the middle of the *GLABROUS1* gene. It is strongly suggested that this mutation is the cause of the trichome loss.

Tottori line of *A. lyrata* is a tetraploid, and accordingly, it had two *GLABROUS1* homologues. Both of them have frameshift mutations: one has an insertion of an adenine residue in the middle of the gene, and the other has 19 bp deletion just after the start codon.

Thus, the inactivation of the *GLABROUS1* gene occurred three times independently, resulting in the loss of trichome twice. Our finding is a good example of parallel evolution. Moreover, evolution by gene loss may be a common mechanism to generate diversity.

350 A Genetic Analysis of Trichome Density in *Arabidopsis thaliana*: A QTL Approach

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The near ubiquity of trichomes (plant hairs) among plant taxa suggests a strong functional role in nature for them. As such, trichomes have long been studied by agronomists, ecologists, systematists, and more recently developmental biologists. Indeed a great deal has been learned about largely qualitative aspects of trichome initiation and development. However, relatively little is understood regarding the more quantitative aspects of trichome development and patterning, for example density and branching, particularly in the context of natural populations. Among wild-collected accessions of *Arabidopsis thaliana*, trichome number, which strongly correlates with density, is relatively normally distributed, ranging over an order of magnitude from fewer than 20 to more than 180 hairs, as measured on third true leaves. Although several glabrous accessions exist, these likely represent individual mutations, rather than genome-wide responses to selection for reduced trichome production. Others' attempts at identifying quantitative trait loci (QTL) affecting trichome density have revealed a single locus, Reduced Trichome Number (RTN), with large effect. This single finding may be the result of the recombinant inbred line (RIL) set used for mapping, as not all RIL sets will be ideally useful for all traits. As such, we are developing four new sets of mapped RILs, initially to be used to map trichome density and eventually to be made publicly available. These sets will be developed from four pairs of accessions not previously used in other RIL sets and are being selected to cover as broad a phenotypic and genetic range as is feasible. To date we have genotyped ~100 accessions at 10 SSLP loci. The range in locus diversity is large, with an average of 17.33 (s.d. = 8.52) alleles per locus. We intend to genotype 15 more loci for all accessions under consideration and to use these data, in combination with phenotype data, to select the four pairs of accessions with which to initiate the RIL sets. Analyses of marker and phenotype data will be presented.

351 Identifying the genetic causes of the evolution of rosette flowering in Brassicaceae: Did the *LEAFY* gene play a role?

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The identification of the genes responsible for phenotypic evolution is a major task in evolutionary developmental genetics. The idea of plant phenotypic evolution occurring via changes of genes of large effect has now become generally accepted. If correct, it should be possible to identify single genes that have played a major role in the origin of an evolutionary novelties. In *Arabidopsis*, and most Brassicaceae, floral induction results in stems elongating to form an erect inflorescence with numerous flowers that lack subtending leaves. However, three independent lineages of Brassicaceae have switched from producing inflorescences to producing flowers in the axils of rosette-leaves. One hypothesis we are currently testing is that, in one or more of these lineages, the evolution of rosette flowering arose via expanded expression of the floral meristem identity gene *LEAFY* (*LFY*). It has been shown previously that constitutive expression of *LFY* in *Arabidopsis* can result in the formation of rosette-flowers. Also, one of rosette-flowering species, *Jonopsidium acaule*, shows an expanded zone of *LFY* expression when tested by in situ hybridization. Therefore, we are using a transformation approach to evaluate whether *LFY* might be the key genetic difference between three rosette-flowering species and their inflorescence-bearing relatives (as represented by *Arabidopsis*). Clones of *LFY* orthologs with their cis-regulatory regions were obtained from the rosette-flowering plants using a genome-walking method. We are examining the effect of the exogenous genes in both mutant and wild-type backgrounds by transforming *LFY/lfy* heterozygous *Arabidopsis* and analyzing the transgenic progeny. We will also introduce GUS-reporter constructs to examine the ability of the 5' regulatory regions from rosette-flowering species to drive expression in the *Arabidopsis* genetic background. If the rosette-flowering species show expanded zone of *LFY* expression and if transformants show elements of rosette flowering then we would have evidence that *LFY* regulation played a role in the evolutionary loss of the inflorescence. A genomic approach using microarrays is currently under consideration to facilitate the identification of other critical genes for the evolution of rosette-flowering.

352 CPR5, a novel regulator of cell death

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The *cpr5* mutant (constitutive expresser of pathogenesis related genes) is characterized by constitutive resistance to the virulent pathogens *Pseudomonas syringae* pv. maculicola ES4326 and *Peronospora parasitica* Noco2. It has elevated levels of salicylic acid (SA), a chemical whose biosynthesis has been shown to be induced by and required for systemic acquired resistance (SAR). In addition, it has several developmental phenotypes: reduced trichomes, reduced cell expansion and a loss of apical dominance. Importantly, it also spontaneously develops lesions that have been demonstrated to mimic the hypersensitive response (HR).

The *CPR5* gene was cloned using a map-based approach and the full-length genomic sequence complemented all *cpr5* mutant phenotypes. The gene encodes a novel 62 kD protein with five predicted transmembrane domains. The full-length cDNA was obtained through RACE and expressed in *cpr5-1*, *cpr5-2* and ColWT plants under the control of the CaMV 35S promoter. Expression of the *CPR5* cDNA in the mutant background rescued the morphological and disease resistance phenotypes. The cDNA complemented lines showed levels of susceptibility to virulent pathogens comparable to that of ColWT. However, in the WT background, over-expression of the *CPR5* cDNA resulted in abnormal developmental and cell death phenotypes. These transformed plants displayed a loss of apical dominance and early senescence of the cauline leaves. Cosuppressed lines displayed a more severe phenotype then either *cpr5-1* or *cpr5-2* mutant allele. These data suggest that neither mutant is the result of a complete loss of function of the CPR5 protein and that the regulation of *CPR5* expression is important to normal development.

353 Identifying components of stress/disease signalling pathways leading to Arabidopsis GST6 gene expression

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Glutathione S-transferases (GSTs) detoxify a range of xenobiotics by conjugation to glutathione and play a role in protection of tissues against oxidative damage. Expression of the Arabidopsis GST6 gene is induced by auxin, salicylic acid (SA) and H_2O_2, implicating this gene in plant stress/defence responses. This regulation is mediated in part by a 20bp ocs promoter element, although other elements are also involved (Chen and Singh, 1999). The plant ocs/as-1 element was initially characterised in the Agrobacterium octopine synthase (OCS) gene promoter and the CaMV 35S promoter, where it has apparently been exploited by the pathogen for expression within infected plant cells. Expression of an ~800 bp GST6 promoter fragment in response to SA and H_2O_2 occurs primarily in the root, making this an ideal system for exploring the less studied below-ground responses of plants to stress and disease. GST6::luciferase and ocs-element::luciferase reporter lines are being used in screens for mutants in the SA and H_2O_2 signalling pathways. Root expression of luciferase from seedlings grown on agar plates is visualised using a cooled CCD camera system, allowing a high throughput, non-invasive mutant screen. Following EMS mutagenesis, a large number of M2 plants have been screened and several putative mutants have been isolated. Progress with screening and phenotype characterisation will be presented.

Chen, W. and Singh, K.B. (1999) Plant J. 19:667-677.

354 Genetic Analysis of Disease Resistance Mediated by the EDR1 MAPKK Kinase

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EDR1 was identifed as a possible regulator of defense responses through a screen for Arabidopsis mutants with enhanced disease resistance (Frye and Innes, 1998). The *edr1* mutation confers enhanced resistance to both *Erisyphe cichoracearum* (powdery mildew) and *Pseudomonas syringae* (bacterial speck). Mutant plants are able to prevent the formation of *E. cichoracearum* conidiophores and also form large lesions uncharacteristic of this interaction. Because of these phenotypes, it is thought that *EDR1* acts a negative regulator of defense responses. *EDR1* encodes a putative MAP kinase kinase kinase, showing homology to *CTR1*, a negative regulator of ethylene responses (Frye et al., 2001). In order to understand how *EDR1* may negatively regulate defense responses, we are currently conducting two experiments to identify components that are part of or interact with the *EDR1* pathway. First, we are mapping the genetic location of an *EDR1* enhancer, *eed1*. Mutants in *EED1* show a necrotic phenotype similar to senesence that begins quite early in the plant's life cycle. This phenotype begins in the oldest leaves and eventually spreads to all the leaves, indicating this is a developmentally regulated phenomenon. These plants remain fertile and produce seed, however. In a population derived from a backcross to wild-type plants, the *eed1* mutant phenotype segregated 1/16, suggesting that it is dependent upon the edr1 mutation. Using a combination of markers that are linked to *EDR1*, we have confirmed that plants displaying this necrotic phenotype are homozygous for *edr1*. In addition to this approach, we are also using Yeast Two-Hybrid analysis to uncover downstream components. Library screening and directed experiments using putative MAPKKs will be used in this analysis to identify the possible target(s) of *EDR1*. Identification of a MAPKK would confirm that *EDR1* functions at the top of a classical MAP kinase cascade.

355 Transgenic Expression of the Bacterial HR Elicitor Harpin Activates the SA-dependent and Jasmonate/Ethylene-dependent Defense Pathways in Arabidopsis.

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Harpin is a protein elicitor secreted by a number of bacterial pathogens of plants. Infiltration of harpin ($HrpN_{Ea}$) from *Erwinia amylovora* into leaves causes a hypersensitive response. Topical application of purified $HrpN_{Ea}$ on plants induces systemic acquired resistance (SAR) and resistance to a variety of bacterial, viral, and fungal pathogens, and increases growth. The $HrpN_{Ea}$ gene was stably introduced into Arabidopsis to evaluate whether effects of topical application can be reproduced by transgenic expression of harpin and to gain insight into mechanisms of harpin action. To test the effects of extracellular vs. intracellular targeting of harpin protein, expression constructs were made with or without a signal sequence fusion to the harpin N-terminus. The nopaline synthase promoter (NOS) was used to provide constitutive harpin expression. Although harpin is an HR elicitor, constitutive expression did not have detrimental effects on plant growth or health. Topical application of harpin induces expression of the SA-dependent pathway marker gene PR1 and the jasmonate/ethylene-dependent pathway marker gene PDF1.2, and inhibits infection by *Pseudomonas syringae* pv. tomato DC3000. Non-segregating third generation transgenic lines were examined for expression of PR1 and PDF1.2. In lines expressing harpin with the N-terminal signal sequence, both PR1 and PDF1.2 were expressed constitutively. PR1 and PDF1.2 mRNAs were undetectable in lines expressing harpin lacking the signal sequence indicating that the site of action for harpin-mediated induction of these defense pathways is extracellular. Independent T3 lines were resistant to DC3000 infection relative to wild type plants. Increased rosette size, root mass, and accelerated bolting were displayed by independent lines expressing harpin. Our initial results indicate that expression of harpin in transgenic plants elicits defense and growth responses that reproduce those obtained from topical application of harpin.

356 Towards the identification of plant host genes which contribute to compatible Arabidopsis-*Peronospora parasitica* interaction

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While the molecular bases of defense reactions in plants against pathogens are well studied, little is known about host factors involved in compatible plant-pathogen interactions. Plant genes that contribute to successful growth of compatible pathogens are potentially interesting because they may reveal important details about interactions between hosts and pathogens, as well as identify novel targets for the development of strategies to protect plants against disease. To identify host genes involved in compatible plant-pathogen interactions, we used genetic and molecular techniques to examine Arabidopsis after infection with the obligately biotrophic oomycete *Peronospora parasitica*. For our genetic screen, we mutagenized and screened Ws-0 *nim1-1* plants after inoculation with the *P. parasitica* isolate Emwa1, which is pathogenic on Ws-0, and highly virulent on defense-suppressed *nim1-1* plants. We have thus far discovered two mutants with severely reduced pathogen growth. Both show microscopic pathogen-induced lesions, but without constitutive or induced expression of the defense genes *PR-1* and *PDF1.2*. Further, one of the mutants shows compatibility to the bacterial pathogen *Pseudomonas syringae* pv. *syringae* DC3000, suggesting that its phenotype is specific to compatible *Peronospora*. In a companion molecular study, we used cDNA-AFLP to identify ten plant genes induced or repressed during compatible pathogen growth. We reasoned that the expression of some of these may be manipulated by the parasite for its own benefit. In follow-up studies, we used northern analyses to monitor the expression patterns of the genes after plants were treated with biotic and abiotic elicitors of defense. Of seven compatible-*Peronospora*-specific genes, three are induced and four are repressed. Three others, including an Arabidopsis homolog of the barley *Mlo* gene, are up-regulated both by compatible *Peronospora* growth and various other inducers. Together, these results suggest that we may have identified a set of host genes whose expression is altered by compatible pathogen growth. Functional tests are underway to determine whether these genes are required for successful compatible pathogen growth.

357 Crosstalk of phytochrome signaling with the SA-perceptive pathway in Arabidopsis

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The interference of phytochrome signaling with the SA-signal transduction pathway has been investigated in *Arabidopsis* using single and multiple mutants affected in light perception (phyA- and phyB- deficient) and light-signal processing (*psi2,* phytochrome signaling). The induction of *PR1* by SA and functional analogs has been found to strictly correlate with the activity of the signaling pathway controlled by both phyA and phyB photoreceptors. In darkness as well as dim light, and independently of a carbohydrate source, SA-induced PR gene expression as well as the hypersensitive response to pathogens (HR) are strongly reduced. Moreover, the initiation of HR also exhibits a strict dependence upon both the presence and the amplitude of a phytochrome-elicited signal. The growth of a compatible race of bacterial pathogens (*Pseudomonas syringae pv maculicola*) was enhanced in *phyA-phyB* and decreased in *psi2* mutants. While functional chloroplasts were found necessary for the development of an HR, the induction of PR genes was strictly dependent on light, independently of functional chloroplasts. Taken together, our data demonstrate a crosstalk between the light-induced signaling pathway and the pathogen/SA-mediated signal transduction route. These data will be presented in a Boolean formalism corresponding to the interface of a digital program that allows qualitative computer simulation.

358 Mutation of an *Arabidopsis* Copine Gene Triggers Cell Death and Increased Disease Resistance

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We have identified and characterized an *Arabidopsis* copine mutant (*cpn1-1*) with improved resistance to virulent strains of *Pseudomonas syringae* pv.tomato and *Peronospora parasitica*. The copines are a newly identified protein class that is highly conserved from nematodes to plants to humans and they may be involved in calcium signaling and membrane trafficking. The phenotype of *cpn1-1* is strongly dependent on relative humidity. The *cpn1-1* mutant exhibits constitutive defense responses and increased disease resistance when grown under low humidity conditions (LH) (40-45% relative humidity) but behaves like the wild type when grown under high humidity conditions (HH) (75-80% relative humidity). LH-grown *cpn1-1* mutants are stunted and have curled leaves with minute necrotic lesions that resemble the hypersensitive cell death response and constitutively display biochemical and molecular markers of defense. This is the first humidity-dependent lesion mimic mutant reported and the first report that a copine may play a role in defense signaling. Our analyses suggest that the *CPN1* gene encodes a repressor of cell death and defense.

359 Genetic dissection of *dnd1*-mediated resistance

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The Arabidopsis *dnd1* mutant exhibits high levels of resistance to bacterial, fungal, and viral pathogens and exhibits an inability to elicit the HR. To further understand the genetic mechanisms that regulate constitutive resistance and suppression of HR cell death in *dnd1*, we are carrying out epistasis experiments with other Arabidopsis defense mutants. Separately, we are performing a screen to identify genetic suppressors of *dnd1*. Initial work with double mutants has monitored the dwarf plant size, which is related to elevated levels of salicylic acid. We are presently performing bacterial growth and HR assays.

Plant size phenotypes suggest an additive effect between *cpr5* and *dnd1*, and between *dnd1* and *dnd2*. The double mutants from these crosses exhibit a superdwarf appearance (smaller than both parents), suggesting that these mutations activate defenses by initially disparate mechanisms rather than as part of the same pathway. In contrast to these results, introduction of *ndr1* into a *dnd1* background partially relieves the dwarf phenotype of *dnd1*, suggesting that *ndr1* is an important mediator of at least part of the *dnd1*-elicited phenotype. Surprisingly, a *dnd1 npr1* mutant does not relieve dwarfism in *dnd1*. This double mutant suggests that *dnd1* acts via pathways that are independent of *npr1*. In further support of this hypothesis, *dnd1 npr1* plants exhibit strong constitutive activation of a β-glucanase:GUS gene fusion. Additional work on these epistasis experiments and our *dnd1* suppressor screen will be reported.

360 Developmental Defects Associated with TuMV P1/HC-Pro Expression in Arabidopsis

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Turnip Mosaic Potyvirus (TuMV) causes severe morphological and developmental defects in the C24 ecotype of *Arabidopsis thaliana*. Infected leaves are stunted, deeply serrated and display a mosaic pattern. The bolts are stunted and curled. TuMV-infected arabidopsis flowers display several developmental defects, including narrow sepals, split carpels, and aborted anthers. Some but not all flowers on infected plants have homeotic-like defects, such as staminated sepals and carpellated petals. C24 plants systemically infected with TuMV are sterile. Interestingly, many of these defects are caused by P1/HC-Pro, an RNA silencing suppressor required for virus replication and systemic movement through plants. Transgenic arabidopsis expressing the 35S-TuMV P1/HC-Pro gene have the same developmental defects as the TuMV infected plants. The role of P1/HC-Pro silencing suppression activity on these developmental malformations is under investigation.

361 Hormone responses in oxidative stress

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Ozone exposure has been shown to elicit many of the defense responses of plants, particularly those induced by pathogens, and therefore, can be used as a tool to study defenses common to various stresses involving some form of oxidative stress. Many of the plant defense responses to both biotic and abiotic stresses seem to be regulated by only a small number of signal transduction pathways mediated by ethylene, jasmonic acid (JA), and salicylic acid (SA). To elucidate the role of these signaling components in oxidative stress, we exposed *Col-0*, ethylene insensitive *ein2*, JA insensitive *jar1*, SA insensitive *npr1*, SA degrading NahG, and ozone sensitive *rcd1* (Plant Cell **12**:1849) *Arabidopsis* plants to 250 ppb ozone for 6 hours. Hormone responses were studied by a cDNA macroarray analysis of 127 stress- and signaling-related genes, and by analysing the ozone induced changes in concentrations of ethylene, JA and SA in different mutants by GC-MS. We used cluster analysis for macroarray results to reveal groups of coregulated genes during oxidative stress and to elucidate hormonal regulatory circuits. The results indicated that cell death triggered by ozone is regulated by ethylene production and sensitivity, and the antagonistic interaction between SA and JA, and suggest the involvement of an additional signaling component, ABA.

362 Identification and Characterization of Arabidopsis Mutants with Altered Susceptibilities to Turnip Mosaic Potyvirus

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To better understand the interactions required between potyviruses and Arabidopsis for infection a model host-virus system was established using *Arabidopsis thaliana* and turnip mosaic potyvirus (TuMV). Infection of Arabidopsis with TuMV causes severe developmental defects that can be used as a phenotypic marker to rapidly identify individuals that are systemically infected. A mutant screen was carried out to identify mutants with altered susceptibilities to TuMV. Eleven mutants were recovered from approximately 159,600 M2 plants derived from 14,000 EMS-mutagenized families. Five of the mutants have been further characterized, revealing two phenotypic classes. Mutants in the first class fail to support systemic infection by either TuMV or the related potyvirus TEV-GUS. Mutants in the second class exhibit a slow or limited systemic infection with both TuMV and TEV-GUS. Both classes of mutants support systemic infection of the unrelated virus turnip crinkle carmovirus. Thus, the mutants were designated *loss-of-susceptibility to potyviruses (lsp)*. Further genetic characterization of the mutants has identified two independent complementation groups that map to different regions of chromosome 5.

363 Analysis of the *Arabidopsis*-powdery mildew interaction: cloning and characterization of mildew-induced lesion mutants.

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The *mil* (mildew-induced lesions) mutants were identified in a screen looking for increased resistance to the fungal pathogen powdery mildew (*Erysiphe cichoracearum*). These resistant mutants abnormally form necrotic lesions ~7 days after inoculation. These mutants fall into at least 7 complementation groups. The lesion and resistance phenotypes of the mutants are being examined using a wide range of biotic and abiotic stresses. Three mutants that form lesions specifically in response to pathogens are being pursued for further characterization and cloning. By cloning and characterizing the *mil* mutants we hope to discover novel components of the signal transduction pathways leading to defense responses.

364 Characterization of dll1: an Arabidopsis gain-of-function mutant that spontaneously develops lesions mimicking cell death associated with disease.

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A salient feature common to both compatible and incompatible plant-pathogen interactions is host cell death. While much is known about the mechanisms regulating host cell death during incompatible interactions, very little is known about the mechanisms regulating host cell death during compatible interactions, especially the role of plant genes in host-determined susceptibility. Here we describe the characterization of a novel gain-of-function Arabidopsis mutant, *dll1* (disease-like lesions), which spontaneously develops lesions mimicking bacterial speck disease. Following lesion formation, *dll1* plants constitutively express biochemical and molecular markers associated with pathogen infection. Despite the constitutive expression of these defense-related markers, *dll1* is not able to repress the growth of virulent pathogens. However, *dll1* displays normal HR in response to avirulent pathogens, thus indicating that the gene-for-gene mediated resistance response is functional in these plants. Unlike wild type Col-0, *dll1* supports the growth of hrp-mutant strains of P.syringae, thus suggesting that *dll1* intrinsically expresses many of the cellular processes that are required for pathogen growth. Through epistasis analyses, we demonstrate that salicylic acid, NPR1, and ethylene signaling regulate the formation of disease-like lesions in *dll1*. These results suggest that a) significant overlap exists between the signaling pathways leading resistance- and disease-associated cell death and b) host cell death during compatible interactions, at least in part, is genetically controlled by the plant and DLL1 positively regulates this process. Further characterization of the *dll1* mutant and the cloning of the corresponding gene should help increase our knowledge of the cell death signaling pathways activated during disease.

365 alpha-dioxygenase: a role in controlling cell death

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We have identified a new pathogen induced enzyme of the oxylipin pathway, which as lipoxygenase, catalyze primary oxygenation of fatty acids. Biochemical studies revealed that this protein is an alpha-dioxygenases (alpha-DOXs) which give rise to the formation of a new group of lipid-derived oxylipins. The expression of the Arabidopsis alpha-DOX1 gene is induced in response to both incompatible and compatible bacterial infections. However the level of alpha-DOX1 mRNA and dioxygenase activity appears early and reaches higher values when infection promotes a hypersensitive reaction. Analysis of transgenic alpha-DOX1:GUS Arabidopsis plants revealed that gene expression is confined to necrotic lesions during the hypersensitive response and to the chlorotic area during a compatible interaction. Accumulation of alpha-DOX1 transcripts is impaired in SA-compromised plants and induced by SA and by chemical treatments generating NO, intracellular superoxide or singlet oxygen, three signals reported to act synergically to potentiate host cell death. Transgenic plants with altered levels of alpha-dioxygenase activity react like wild-type plants to a compatible pathogen. On the other hand plants with reduced activity develop a more severe necrotic response than wild-type plants to incompatible bacteria or paraquat treatment, and a milder response when alpha-DOX1 was overproduced. These results suggest that plant alpha-dioxygenases are used to generate lipid-derived signal molecules for a process that protects plant tissues from cell death. A second alpha-dioxygenase Arabidopsis gene, alpha-DOX2, is presently being characterized. Mutation of a tomato homolog provokes a severe alteration in plant development, suggesting that the enzyme encoded by the Arabidopsis alpha-DOX2 gene might catalize a different enzymatic reaction than that characterized for the alpha-DOX1 protein. alpha-DOX2 enzyme functionality studies are in progress.In addition subcellular localization of both enzymes is being investigated by using GFP chimeric constructs

366 Genetic analysis of disease susceptibility in the *Arabidopsis thaliana - Peronospora parasitica* interaction

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The oomycete *Peronospora parasitica* is a biotrophic pathogen of *Arabidopsis thaliana* on which it causes downy mildew. After germination of a spore the plant is penetrated and invading hyphae are formed. The hyphae grow intercellularly and haustoria develop in adjacent plant cells by invagination of the plasma membrane. The haustorium is believed to play an important role in reallocation of nutrients and in cell-cell communication. In our research we intend to find host genes that are important for susceptibility to *P. parasitica* infection, in particular genes required for the development and functioning of the haustorium. We have EMS-mutagenised seeds of the highly susceptible *A. thaliana* Landsberg *erecta* mutant *eds1-2*. From ~3500 M2 families we have isolated 24 *dmr* (for downy mildew resistant) mutants with reduced susceptibility to *P. parasitica*, i.e. showing strongly reduced sporulation of the pathogen. One class of mutants shows an enhanced resistance phenotype with elevated levels of defense gene expression (e.g. *PR-1*). We are currently characterizing a set of *dmr* mutants in which *P. parasitica* infection is quickly arrested but haustoria are still being formed. These mutants do not show enhanced *PR-1* expression and no cell death is observed after infection. In some of these mutants the haustoria have an abberant form and/or are surrounded by callose. We will report on the analysis of disease susceptibility of *dmr* mutants to other pathogens and on our effort to map the corresponding genes.

367 lra2 Mutants Specifically Affect RPM1 Signaling

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Recognition of a pathogen is important for the plant to be able to mount an effective response. While many genes necessary for recognition of pathogens and subsequent signaling have been identified in *Arabidopsis*, the exact mechanism by which pathogen perception and concomitant signaling occurs is still not well understood.

In our lab we study *RPM1*, which is unique among resistance genes in that it can recognize the products of two different bacterial genes, *AvrRpm1* and *AvrB*. Using transgenic plants containing *AvrRpm1* under an inducible promoter, we have identified a new mutant, which has lost the ability to recognize AvrRpm1. We have designated this mutant *lra2*, for loss of recognition to AvrRpm1. This mutant is also susceptible to DC3000 expressing AvrB, but not to any other pathogen tested.

We found four alleles of this mutant, which all show nonallelic noncomplementation when crossed to plants lacking functional *RPM1*. Thus the F1 progeny of this cross all lack recognition of AvrRpm1. This shows a genetic interaction between these two genes. We will discuss the above data as well as the current state of our mapping of this gene.

Research supported by NSF grant IBN-9724075.

Postdoctoral Fellowship from the Spanish Ministry of Science and Education to P.T.

368 Functional interaction of CBL-type calcium sensor proteins and protein kinases in various signaling cascades

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Calcium signals in plant cells are elicited by a variety of stimuli such as hormones, light and stress factors. We have recently described a new family of calcineurin B-like (CBL) calcium sensor proteins from *Arabidopsis* and identified a specific group of serine-threonine protein kinases as targets of these sensor proteins. A detailed analysis of protein-protein interaction revealed a conserved 24 aa domain within the C-terminal region of these kinases as necessary and sufficient to mediate interaction with CBL proteins. This domain defines 24 kinases from *Arabidopsis* as targets of CBL sensor proteins indicating that the cellular processes mediated by these kinases are subject to regulation by calcium signaling. Expression studies indicate a function of these kinases in numerous signaling processes. Comparative CBL-kinase interaction studies suggest differential interaction affinity as one of the mechanisms generating the temporal and spatial specificity of calcium signals within plant cells. Thus various combinations of different CBL/kinase proteins can form a complex network that connects extracellular signals to defined cellular responses. Results of the molecular and genetic analyses revealing the function of these proteins and mechanisms leading to their temporal and spatial specificity in signaling will be presented.

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369 The interaction of the obligate biotrophic parasite *Plasmodiophora brassicae* with *Arabidopsis thaliana*.

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The obligate biotrophic parasite *P. brassicae* induces galls in the roots of the model organism *A. thaliana*. The ecotype Tsu-0 of *A. thaliana* revealed to be resistant to *P. brassicae* isolate eH. The resistance reaction is accompanied by an hypersensitive re-action. Infected cells were surrounded by necrotic boundaries and thereby the infection area is encapsulated. This resistance has been shown to be pathotype-specific, dominant and monogenically inherited. The ecotypes Ze-0, Ta-0 and RLD also show resistance phenotypes and tests for allelism indicate that these ecotypes carry alleles of the gene RPB1. Using a backcross mapping population from the cross Tsu-0 (RPB1/RPB1) x Cvi-0 (rpb1/rpb1) consisting of app. 4250 plants we established a high resolution map around the RPB1 gene and mapped the gene on one large BAC and on the overlapping region of two small BAC-clones. Six gene candidates were localised in the region of RPB1. Transformation of susceptible lines with gene candidates has been performed to obtain the biological proof of gene function.

370 Identification of Genes Involved in the Response to Water-Deficit Stress

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Two different methods are being used to select/screen for Arabidopsis genes that are expressed and functioning in response to water-deficit stress. Mutants were selected using an allyl alcohol strategy. A line homozygous for a construct containing the *le25* promoter (a gene from tomato that requires elevated levels of ABA for expression) fused with ADH in an *adh* null background was EMS mutagenized. Plants were grown in pools and the resulting seeds were germinated on plates without stress. Seedlings were transferred to plates conditioned with PEG (low-water potential) in order to provide a water-deficit stress. After a 2 hr allyl alcohol treatment, seedlings were transferred to high-water potential plates for a one-week recovery period. Seedlings that survived this treatment were transferred to soil to set seed. In the secondary screen, seeds from individual plants were tested for proline production in response to water deficit and were retested for allyl alcohol insensitivity. Interestingly, allyl alcohol resistant lines were recovered that had both increased and decreased proline accumulation in response to low-water potential treatment. A second method is being used to identify genes that are expressed in response to a water-deficit treatment. The UC Riverside gene trap lines were screened for altered patterns of GUS expression in response to seedling wilting. Two plates of each line were grown in a high-humidity chamber. To impose stress, one plate was transferred to a low humidity environment and the lid was removed from the plate. This treatment increased ABA levels and induced the expression of known water-deficit-induced genes. After GUS staining, the expression pattern of the control and stressed lines were compared. This method has resulted in the identification of genes and water-deficit-induced gene expression patterns that have not been previously identified using other methods.

371 The absence of flavonoids in the chalcone synthase mutant *tt4* leads to elevated auxin transport and impaired gravitropic bending

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Polar transport of the plant hormone auxin controls many aspects of plant growth and development including gravitropic responses. A number of synthetic compounds have been shown to prevent gravitropism by inhibition of the auxin efflux carrier complex and may act by mimicking endogenous molecules. Specific flavonoids, from the class of secondary plant metabolic compounds whose synthesis is regulated by a number of environmental factors, have been suggested to be endogenous auxin transport inhibitors based on their *in vitro* activity. Two alleles of the *tt4* mutation in the gene encoding the first enzyme in flavonoid biosynthesis, chalcone synthase, lack flavonoids and have elevated auxin transport. Elevated auxin transport in *tt4* plants can be reversed by growth of plants on naringenin, a flavonoid precursor. A phenotypic examination of *tt4* plants revealed a significant delay in root and hypocotyl gravitropic bending, along with increased root and inflorescence branching as compared to wild-type, all phenotypes consistent with altered auxin transport. The localized accumulation of flavonoids can be identified by use of a dye that becomes fluorescent upon binding flavonoids. Flavonoids are localized to tissues that exhibit gravitropic bending, such as the distal elongation zone of roots. These results suggest that individual flavonoid derivatives function as endogenous regulators of auxin transport, which are specifically active in regulation of auxin transport during gravitropic bending. (This work was supported by NASA grant NAG2 1203 and the NSCORT in Plant Biology at NC State University).

372 Does Em protein expression increase osmotic stress tolerance?

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One of the major events during seed development is the growth and development of the embryo (embryogenesis). Accumulation of late-embryogenesis-abundant (LEA) proteins occurs during the maturation stages of embryogenesis of most angiosperms. . The accumulation of LEA proteins in the embryo has been shown to correlate to increased levels of the phytohormone ABA and increased desiccation tolerance. Group 1 LEA proteins are thought to protect the embryo from desiccation by binding and retaining cellular water. The early-methionine-labeled (Em) protein typifies Group 1 LEA proteins and is the single most abundant protein found in wheat embryos. Em protein has been shown to provide protection against osmotic stress in transformed yeast cells. It was reported that expression of Em enhanced growth in media of low osmotic potential and had no deleterious effects on growth under normal osmotic conditions. This study provides further evidence that Em is involved in cellular protection during water-deficit stress. Our objectives are to determine if system Em expression would enhance salt and water-deficit stress tolerance in plants. Arabidopsis plants were transformed with the wheat Em gene and 3 mutated Em genes plus a GUS control. Transformed plants were selected by germinating seeds on media containing 50µg/ml kanamycin through 2 generations. Transformations were verified by PCR and Southern blot analysis. Seeds of transformed plants were tested for germination under conditions of hyper-salinity. Seeds were sterilized and spread on 1.3% agar plates containing MS minimal organics with 100, 200, 400, 600, or 800 mM NaCl. Plates were placed in 4°C for 3 days and then placed under continuous light at 25°C. Total seed number was counted and percentage germination determined. Transformed plants were also grown in soil and exposed to water-deficit stress. Leaf discs were cut and sampled for water potential, osmotic potential, and water content and tested by ANOVA with PC-SAS. Results of these tests will be presented.

373 A Signal Terminating Gene From Arabidopsis Can Alter ABA Signaling

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The ability to respond to a variety of biotic and abiotic signals is crucial to many organisms. Signals outside the cell can be perceived and amplified at the cell membrane by a variety of signaling pathways, including the inositol 1,4,5-trisphosphate (IP3) pathway. In plants, there is evidence that common signals such as light, gravity and ABA are perceived via IP3 signaling. To determine whether the signal terminating enzymes that hydrolyze IP3 are regulatory in plants, we have identified fifteen putative inositol 5'-phosphatases (5PTases) in the model plant Arabidopsis thaliana. We have characterized the substrate specificity of one of these enzymes, At5PTase1. At5PTase1 can hydrolyze IP3 and IP4 but not PIP2 substrates. Phylogenetic analysis of the other fourteen At5PTases suggests that Arabidopsis contains two groups of 5PTases that differ in their substrate specificity. To discern whether the At5PTase1 gene can terminate IP3-mediated signaling events, transgenic plants overexpressing At5PTase1 were constructed and demonstrated to contain elevated 5PTase enzyme levels and reduced basal IP3 levels. Leaves from wildtype and transgenic plants were stimulated with ABA to test whether overexpression of At5PTase1 altered ABA-induced stomatal closure. Transgenic stomata were shown to be defective in closure, indicating that At5PTase1 overexpression blocks ABA signal transduction. In similar experiments, we examined the expression of genes previously indicated to be stimulated by ABA signaling (kin1 and At5PTase11). Induction of these genes occurs rapidly in wildtype plants (within minutes), but is significantly delayed in At5PTase1 transgenic plants. This indicates that At5PTase1 overexpression can also inhibit the rapid upregulation of gene expression mediated by IP3 signaling. We are currently examining ABA-stimulated IP3 levels in both wildtype and transgenic plants to determine if At5PTase1 overexpression can repress IP3 levels during signaling.

374 Isolation of hydrotropic mutants in *Arabidopsis thaliana*

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The survival of a plant depends upon the capacity of root tips to sense and move towards water and other nutrients in the soil. The primary site for perception of underground signals is the root cap. Roots from all plants respond to moisture gradients (hydrotropism); however, we still do not know how this tropism works. To study the molecular genetic mechanisms controlling hydrotropism, we have developed a screening method for isolating hydrotropic mutants in *Arabidopsis thaliana*. We have isolated a novel EMS line of *A. thaliana* mutants that do not respond to hydrotropism from M2 plants. We have also isolated 1 EMS mutant that responds more rapidly to the hydrotropic stimulus. The phenotypes of the *A. thaliana* lines affected in hydrotropism (*noh*, *n*ot *h*ydrotropic; *suh* or *su*per-*h*ydrotropic) have been conserved for five generations after M2. Genetic analysis showed that the noh and suh mutations are monogenic and recessive. Both *noh*and *suh*roots responded to gravity and touch stimuli. *Noh* mutants show a slight stimulation of root growth in the presence of an auxin transport inhibitor and absicic acid but no differences in root growth were seen when auxin, ethylene, and cytokinin were added to the medium. Detailed characterization of the *noh* phenotype showed a shift in the characteristic organization of the wild type root tip while *shu*root tip morphology is similar to wild type roots. These results show that hydrotropism is amenable to genetic analysis in *A. thaliana*. Molecular examination of these new hydrotropic mutants will help to elucidate the mechanisms that allow a plant to perceive and respond to hydrotropism.

375 Biochemical properties of a novel isoform of plant peroxiredoxin

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A cDNA encoding a newly identified isotype of peroxiredoxin (Prx) was isolated from a chinese cabbage flower bud cDNA library and designated CPrxII. The predicted amino acid sequence of CPrxII has no conserved cysteine, peptide domain, or signal sequence present in most of the 2Cys-Prx subfamily members. Database searches using the predicted CPrxII amino acid sequence revealed no substantial homology to other proteins with the exception of the yeast type II Prx with which CPrxII shares 27.8% sequence identity. However, the CPrxII shows no immuno cross-reactivity to antiserum of the yeast type II Prx, and vice versa. Southern analysis using the cDNA insert of CPrxII revealed that it consists of a small multigene family in chinese cabbage genome. The recombinant protein of CPrxII expressed in E. coli migrates as a dimer in a nonreducing SDS-polyacrylamide gel and as a monomer in a reducing condition. Recombinant CPrxII was able to protect glutamine synthetase from inactivation in a metal catalyzed oxidation system and to reduce H2O2 with electrons provided by thioredoxin. This specific antioxidant activity of CPrxII was about 6 fold higher than that of 2Cys-Prx of the same plant. In contrast to 2Cys-Prx, which is predominantly expressed in leaf tissue of cabbage seedlings, CPrxII is highly expressed in root tissue as revealed by Northern and Western blot analyses.

376 Regulation of metal uptake in Arabidopsis

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In response to iron deprivation, strategy I plants induce both ferric chelate reductase activity and ferrous iron transport activity. We now have in hand genes that encode ferric chelate reductase (*FRO2*) and a ferrous iron transporter (*IRT1*) in Arabidopsis. *FRO2* is predicted to encode a plasma membrane-bound enzyme belonging to a family of flavocytochromes that transport electrons across membranes, while *IRT1* encodes an integral membrane protein that belongs to the ZIP family of metal transporters. IRT1 is known to transport iron, zinc, manganese and cadmium. Because iron is both essential and potentially toxic, the uptake of iron must be carefully regulated; we have taken a number of approaches to study the regulation of iron uptake. Both *FRO2* and *IRT1* are expressed in the roots of iron-deficient plants. Time course experiments demonstrate that *FRO2* and *IRT1* transcript levels are regulated coordinately in response to iron, zinc and cadmium, suggesting that regulation of the two genes is mediated by common cis- and trans-acting factors. Transgenic plants engineered to overexpress IRT1 revealed that IRT1 is subject to post-transcriptional regulation by iron and zinc. Experiments in yeast suggest that IRT1 is regulated post-translationally via regulated endocytosis in response to high zinc levels, like the yeast high affinity zinc transporter, ZRT1 (Gitan and Eide, Biochem J. 346:329-336). Thus, it appears that regulation of IRT1 occurs at two levels and in response to multiple metals. 35S-*IRT1* plants show an enhanced sensitivity to cadmium under iron-deficiency conditions, presumably due to elevated levels of IRT1 protein in iron-deficient roots. The enhanced sensitivity of the 35S-*IRT1* plants to cadmium has allowed the identification of cadmium resistant mutants. Several of these mutants display alterations in IRT1 protein accumulation and presumably define factors involved in the regulation of IRT1 accumulation. Analysis of FRO2-*GUS* transgenic plants reveals that FRO2 is expressed at high levels in the epidermal cells of lateral roots in iron-deficient plants. Finally, we have constructed 35S-*FRO2* transgenic plants; analysis of these plants will be presented.

377 Structure of a flavin-binding plant photoreceptor domain: Insights into light-mediated signal transduction

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Phototropin, a major blue-light receptor for phototropism in seed plants, exhibits blue light-dependent autophosphorylation and contains two light, oxygen, or voltage (LOV) domains and a serine/threonine kinase domain. The LOV domains share homology with the PER-ARNT-SIM (PAS) superfamily, a diverse group of proteins involved in cellular signaling. Each LOV domain non-covalently binds a single FMN molecule and exhibits reversible photochemistry in vitro when expressed separately or in tandem. We have determined the crystal structure of the LOV2 domain from the phototropin segment of the chimeric fern photoreceptor phy3 to 2.7 Angstrom resolution. The structure constitutes a novel FMN-binding fold that reveals how the flavin cofactor is embedded in the protein. The single LOV2 cysteine residue is located 4.2 Angstrom from flavin atom C(4a), consistent with a model in which absorption of blue light induces formation of a covalent cysteinyl-C(4a) adduct. Diffraction data collected on phy3 LOV2 crystals under continuous illumination by white light reveals a steady-state structure in which the cysteine side chain has moved away from its ground state position to a new position centered over C(4a) of the flavin ring. Concomitant with this cysteine side chain motion is a slight tilting of the flavin ring. This steady-state LOV2 structure has a spectrum in the crystal corresponding to a long-lived metastable intermediate in the LOV2 photocycle and may act as the signalling state of LOV domains during the phototropic response. Interestingly, residues that interact with FMN in phy3 LOV2 are conserved in LOV domains from phototropin of other plant species and from three proteins involved in the regulation of circadian rhythms in Arabidopsis and Neurospora. This conservation suggests that all exhibit the same overall fold and share a common mechanism for flavin binding and light-induced signaling.

378 The *sob* mutants: activation tagged suppressors of *phyB-4*

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Loss-of-function genetic screens in *Arabidopsis* have been used to identify roles for the photomorphogenic photoreceptors phytochromes (phy) and cryptochromes (cry) during seedling deetiolation and plant development. However, given the complexity of photomorphogenic development and the relatively few genes in these processes that have been identified in loss-of-function mutant screens, it is clear that this approach will not allow the genetic elucidation of all the signaling components downstream of phytochromes and cryptochromes. Since loss-of-function screens may not identify redundant or essential components in these pathways, we are employing a gain-of-function activation-tagging screen for suppressors of the long hypocotyl phenotype of the weak allele *phyB-4*. *phyB-4* plants are transformed via *Agrobacterium tumefaciens* with a T-DNA containing four copies of enhancer regions from the CaMV 35S promoter, a kanamycin resistance gene for plants, an *E. coli* origin of replication and bacterial ampicillin resistance. This screen has led to the identification and cloning of at least one new suppressor of *phyB* (dominant), *sob1-D*. The *sob1-D* mutant has a long hypocotyl in the dark, indicating that this plant is capable of normal etiolated growth. *sob1-D* mutant seedlings are hyper-responsive to far-red, blue and white light. The hyper-response to blue and far-red light requires cry1 and phyA respectively, placing this mutation downstream of these two photoreceptors. The *sob1-D* mutation fully suppresses a null *phyB* allele in white light indicating that this suppressor does not require phyB activity to be functional, and genetically placing this as a bypass suppressor of *phyB*. Cloning of the *sob1-D* mutation indicates that these phenotypes are most likely caused by the over-expression of a transcription factor. We hypothesize that this transcription factor is a point of convergence between phyA and cry1 signal transduction pathways.

379 Using Arabidopsis as a Model to Study the Induction of Herbicide Detoxification Systems by Safeners

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Herbicide selectivity is central to the success of chemical weed control in agriculture. For years, herbicide safeners have been used to enhance the selectivity of weed control in cereal crops. Safeners act predominantly by inducing herbicide detoxifying enzymes such as glutathione S-transferases (GSTs). Safener-induced GSTs can conjugate an herbicide to glutathione (GSH), thereby providing tolerance to that compound. However, little is known about how safeners induce detoxification systems in plants. This investigation seeks to use Arabidopsis as a model to study safener response mechanisms in higher plants. Treatment of Arabidopsis seedlings with safeners resulted in increased GST activity against both the model substrate CDNB and herbicide substrates. GSTs from safener-treated seedlings were purified by GSH-affinity chromatography and displayed by 2-D SDS-PAGE. A 25.6 kD protein that was induced by a number of safeners was identified by MS analysis of trypsin-digest fragments as a novel GST, *At*GSTU2. When expressed in *E. coli*, *At*GSTU2 has high CDNB activity and is able to conjugate several chloroacetamide herbicides at rates similar to those reported for GSTs in sorghum and maize. Based on its high level of activity with CDNB and herbicides, and its abundance in protein extracts, it is likely that *At*GSTU2 is the major contributor to the increase in GST activity following safener treatment. RNA blot analysis confirmed that *At*GSTU2 transcript levels increase in response to safeners that are utilized in cereal crops to increase chloroacetamide herbicide selectivity. RNA blot analysis using cDNA probes representing other Arabidopsis GSTs showed that expression of a number of GSTs is upregulated in response to safeners. However, all safeners do not induce the same profile of GSTs in Arabidopsis, suggesting that multiple pathways are involved in safener regulation of GST expression. Analysis of transgenic Arabidopsis lines overexpressing *At*GSTU2 as well as reporter gene constructs driven by the *At*GSTU2 promoter will add to our understanding of the regulation and function of safener-induced GSTs in plants.

380 Repetitive Action Potentials Induced In *Arabidopsis thaliana* C24

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Communication inside the whole plant is necessary for its adaptation to environmental modifications. Intercellular communication can be achieved via chemicals, hydraulic pressure and electrochemical pathways. The typical self-propagated electrochemical variation is characterized by an action potential (AP). To our knowledge, APs have still not been observed in *A. thaliana*, which is a model organism for basic research in plant molecular and developmental genetics. We propose an experimental method to elicit bioelectrical events in the leaf of this plant and present our results in order to illustrate certain properties of these APs.
Bioelectrical measurements were made on the leaf of *Arabidopsis* with extracellular electrodes located along the midrib. The method, named “prick and drop”, consists in applying a drop of KCl (1 M) solution to a wound made by a prick on the leaf of an intact *Arabidopsis* plant. It was experimented in two different conditions: interruption of light and continuous light. Both induce effective spike responses >90 % efficiency), known as repetitive action potentials (RAP). However the calculated appearance of the APs was different in the two experimental conditions, in the light interrupted experiment, the dark transition gave lower but more homogenous amplitudes. We also observed two kinds of spikes with this “prick and drop” method: APs that propagate rapidly and graded potentials with a large wave and a slow propagation (0.3 ± 0.1 mm.s^{-1}). Our experiments inspired from the results of RAP observed in *C. conicum*, show clearly the existence of APs in *A. thaliana* by setting up an efficient and easily reproducible method.

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381 Multiple Photosensory Systems Coordinately Regulate Blue-Light-Mediated Hypocotyl Growth Inhibition

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The hypocotyls of etiolated seedlings grow rapidly in darkness. Upon illumination with blue light, dark-grown seedlings exhibit a membrane depolarization and virtual arrest of hypocotyl growth within 30 s. Because these responses occur almost immediately and are not affected by red light, they are particularly applicable to studies of photoreceptor action in early blue-light signaling. Our laboratory has used electrophysiological and high-resolution imaging techniques to describe the kinetics of light-induced depolarization and of growth inhibition in Arabidopsis seedlings. We have analyzed a series of photoreceptor mutants and have monitored their effect on anion-channel-mediated depolarization, immediate growth inhibition (0-30 min), and long-term growth inhibition (30-120 min). The high-resolution kinetic analyses in various genetic backgrounds has led to identification of early transient roles for phototropin (nph1), cryptochrome2 (cry2) and phytochromes that are not evident after long-term growth under constant blue light. Phototropin, cryptochrome1 (cry1), cry2, and phytochrome A (phyA) are all required for normal amplitude of membrane depolarization. The nph1 receptor mediates the immediate growth inhibition induced by blue light, and is also required for normal long-term growth suppression. The NPH3 protein (a putative scaffold protein required for phototropism) is not required for nph1-mediated growth inhibition, and therefore represents the bifurcation of the growth suppression and phototropism transduction systems. The cry1, cry2 and phyA receptors are all required for growth inhibition between 30-120 min and act with an identical time course, suggesting they operate through a common mechanism. The phytochrome B (phyB) receptor does not influence depolarization and in contrast to cry1, cry2 and phyA, mediates a positive influence on growth that opposes light-induced inhibition. These high-resolution studies show that during the first 120 min in blue-light growth rate is determined by the influence of multiple photosensory systems, and that the instantaneous rate of growth is the balance between inhibition regulated through cry1, cry2 and phyA, opposed by growth promotion initiated by phyB.

382 A clock-regulated promoter motif in *Arabidopsis thaliana*

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Like many other organisms, plants possess internal biological clocks that help them coordinate internal events with the external environment. This circadian clock allows plants to anticipate the environmental changes that occur every 24 hours, most notably the rising and setting of the sun. The widespread presence of circadian rhythms across taxa suggests that they provide organisms with an adaptive advantage.

In all organisms studied thus far, a transcriptional/translational feedback loop lies at the heart of the circadian oscillator. This central clock often controls output genes at the transcriptional level. Using high-density DNA microarrays, we have recently identified over 450 clock-regulated genes that cycle at the steady state mRNA level in *Arabidopsis*. Promoter analysis of these genes led to the identification of a conserved 9-nucleotide motif found in many clock-regulated genes with peak expression at the end of the subjective day (Harmer et al (2000), *Science* **290**:2110-3). We previously showed that this evening element (EE) is required for conferring circadian rhythmicity on a reporter gene. In the work presented here, we show that the EE itself is sufficient to confer circadian rhythmicity on a reporter gene and investigate transcription factors that bind to it. Through this study, we hope to learn how the circadian clock regulates distinct phases of gene expression.

383 ABI3 mediates induction of AtPer1 in response to treatment with ABA or oxidative stress

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Peroxiredoxins (Prx) belong to a group of antioxidants found in organisms ranging from bacteria to humans, and also in plants. This antioxidant group can be divided into two subgroups, 1-Cys and 2-Cys, according to the number of conserved cystein residues. The 1-Cys Prx *AtPer1* in *Arabidopsis thaliana* and *Per1* in barley are only expressed in the embryo and aleurone layer, the only two tissues surviving desiccation of the seed. These genes are expressed in a dormancy related manner, in that they disappear during germination, but are maintained in dormant seeds. To investigate a suggested role in dormancy maintenance, transgenic *Arabidopsis* plants over expressing 1-Cys protein has been made, and plants with a reduced level of *AtPer1* is under preparation.

Peroxiredoxins have *in vitro* been shown to protect lipids, DNA and certain enzymes against thiyl and oxygen radicals, and both PER1 and AtPER1 have been shown to have antioxidant activity *in vitro*. They have been suggested to protect tissues from reactive oxygen species during desiccation and early imbibition.

Expression of the 1-Cys genes cannot be induced by ABA in vegetative tissue, but have been detected in vegetative tissues of transgenic *Arabidopsis* plants ectopically expressing ABI3. Exposure to oxidative stress like H_2O_2 or hydroquinon (HQ) can also induce expression in 11 days old seedlings in this background. Both the *AtPer1* and *Per1* promoters contain a putative antioxidant responsive element (ARE) and an ABA responsive element (ABRE). A promoter-deletion series of the *AtPer1* promoter fused to GUS has been constructed to investigate the function of these elements, and has also revealed 250 bp of the promoter to be sufficient for activation of the promoter in the presence of ABA and ABI3.

384 Functional studies of ClpB/HSP100 family proteins in Arabidopsis.

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The Hsp100/ClpB family of molecular chaperones are found in bacteria, yeast, certain parasitic protozoans, and plants. These proteins have two essential ATP binding sites and are part of a larger class of AAA+ chaperone-like ATPases that are involved in the assembly, operation or disassembly of protein complexes. Proteins of this family are induced by heat shock, exposure to ethanol or cadmium and during periods of nutrient starvation. The Hsp100/ClpB proteins in plants also accumulate at specific developmental stages such as seed maturation. Three genes, *AtHSP101*, *AtHSP92.7* and *AtHSP98.7* , belong to ClpB/HSP100 family in Arabidopsis. AtHSP101 has been shown to be essential for the development of thermotolerance to high temperature, but to be dispensable under normal growth, despite significant accumulation in seeds. The functions of AtHSP92.7 and AtHSP98.7, which are very similar to AtHSP101 at the amino acid level (75.9 or 65.1% similar, respectively), remain unknown. To understand the biological roles of AtHSP92.7 and AtHSP98.7, we have isolated T-DNA insertion knock-out mutants in these genes by use of sequence-based PCR screening. These knock-out mutants show wild type growth and development under optimal conditions. They also acquire thermotolerance like wild type plants in conditions under which an AtHsp101 knockout mutant fails to develop thermotolerance. This result is consistent with the absence of heat shock elements in the promoter region of the *AtHSP92.7* and *AtHSP98.7* genes. Double mutants of AtHsp98.7, AtHsp101 and AtHsp92.7,AtHsp101 have been generated to test the possible complementation of their activities during normal growth. In addition, the effect of other abiotic stresses such as salt, drought and cold on these mutants and the double mutants is being tested.

385 Genetic Control of Homeostasis in Plant Growth

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Wild type Arabidopsis plants grow to roughly the same size over a wide range of temperatures. Maintenance of plant size over these temperatures requires the *BON1* gene because *bon1* null mutants at 22°C make miniature fertile plants, which have both smaller and fewer cells than the same plant at 28°C. *BON1* is expressed in growing tissues (young leaves and the apical elongation portion of the stem). Moreover, the transcription of *BON1* and *BAP1* (*BON1 Associated Protein*) are regulated by temperature: they have elevated expression when plants are grown at low temperature. These expression patterns suggest that *BON1* has a direct role in regulating cell division and expansion at low temperature.

The BON1 protein is associated with the plasma membrane as shown by fractionation experiments and transient expression in protoplasts. BON1 protein contains a Ca^{2+} dependent phospholipid-binding domain and promotes aggregation of lipid vesicles *in vitro*. BON1 is a member of the copine gene family, which is highly conserved from protozoa to humans. Our data suggest that the copine gene family may function in the pathway of membrane trafficking and at least one member of the family, BON1, is required to maintain membrane function at lower temperature.

386 A pleiotropic *Arabidopsis* mutant with altered root wave pattern

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A screen for seedlings with altered root wave pattern on titled agar surface was conducted in an *Arabidopsis thaliana* population mutagenized by a modified *Ac*/*Ds* system from maize. The mutant *wvc16* was identified and isolated for its compressed root wave pattern. Besides defects in root waving on tilted agar surface, the mutant also exhibits other morphological defects such as curly rosette and cauline leaves, kinked siliques, curly pedicles, and extended petals. In addition, the stems of *wvc16* curl, some of which then uncurl over time. The mutant responds to gravity in a manner comparable to wild type plants. Comparison of root growth rate under the influence of exogenous IAA and 2,4-D at various concentrations between wild type and mutant revealed no significant difference. Using adapter PCR, the *Ds* insertion site was identified within the first predicted exon of a novel and hypothetical gene. Northern blot analysis and RT-PCR results both suggested that the mutant is over-expressing a mutant form of *WVC16*. Genetic, molecular, and physiological studies are being carried out to further characterize the nature of the *wvc16* mutation.

387 Function of Arabidopsis NCED Genes in the Biosynthesis of ABA under Drought Stress

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Neoxanthin cleavage enzyme (NCED) catalyses a key step in the biosynthesis of ABA. We have cloned a cDNA (*VuNCED1*) that encodes NCED from *Vigna unguiculata* (cowpea). The expression level of *VuNCED1* was upregulated by drought stress. (Iuchi *et al*. (2000) Plant Physiol. 123: 553-562) There are at least nine genomic sequences that share relatively high homology with *VuNCED1* in *Arabidopsis thaliana*. Among the putative *AtNCED* genes, *AtNCED3* was expressed significantly under drought condition. The enzyme activity of the recombinant AtNCED3 protein was analyzed to reveal that *AtNCED3* encodes active NCED. In the transgenic plants, expression level of *AtNCED3* was correlated with endogenous ABA level and drought tolerance. Present results indicate that upregulation of the expression of *AtNCED3* is responsible for the accumulation of ABA under drought condition.

388 IDENTIFICATION OF A CYTOCHROME P-450 INVOLVED IN ARABIDOPSIS HIGH CO_2 INSENSITIVE RESPONSE

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As atmospheric levels of CO_2 continue to rise, there is significant interest in the mechanisms by which plants can respond to these changes. To identify specific genes involved in high CO_2 acclimation, we have been screening for Arabidopsis mutants that exhibit a non-wild type response when exposed to elevated (1000 ppm) CO_2 concentrations. One class of mutants that has been characterized are designated CO_2 non-responsive (*cnr*) in that they do not display the typical stress responses of increased anthocyanin production, leaf cupping, and stunted growth found in wild type plants exposed to similar high CO_2 levels. Molecular characterization of one such mutant, *cnr2-1*, indicates that T-DNA inactivation of a cytochrome P450 monooxygenase gene has resulted in the CO_2 insensitive phenotype. As found in other *cnr* mutants, the expression of photosynthetic genes such as *rbcS*, chloroplastic carbonic anhydrase (*ca1*), and *cab* in *cnr2-1* is not significantly reduced by high CO_2 exposure as seen in wild type plants. In addition, this mutant also appears to be more drought tolerant than wild type plants. The impact of this lesion on the photosynthetic characteristics of the mutant, desiccation tolerance, growth on glucose, and a possible link with ABA metabolism will be discussed.

389 Analysis of Arabidopsis *old* mutants allows the construction of a regulatory pathway for leaf senescence

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Leaf senescence is a genetically controlled process that marks the final stage of leaf development. Ethylene is a strong modulator of leaf senescence, but it is not believed to control the process itself, as ethylene does not induce senescence in young leaves, and the leaves of ethylene insensitive mutants do senesce. Only a few genes have been identified that are involved in the regulation of leaf senescence and the mechanism behind the control of leaf senescence is not well understood. We have taken a forward genetics approach in order to identify genes involved in the regulation of leaf senescence. We have isolated several *old* (for onset of leaf death) mutants that show an altered senescence phenotype in response to ethylene treatment. The triple response can still be induced, suggesting that the mutants are not affected in this part of the ethylene signal transduction pathway. Three *old* mutants have been studied in more detail and genetic analysis revealed that the mutations are located on 3 different chromosomes. Epistatic analysis suggests that the affected genes may act in a linear pathway to control leaf senescence.

390 Transcriptional regulation of Phosphate transporters in Arabidopsis

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Transgenic plants expressing the reporter genes under the regulation of phosphate (Pi) transporter (*AtPT1* and *2*) promoters serves as an excellent tool to study the transcriptional regulation of genes during Pi starvation. In this study have analyzed transgenic *Arabidopsis* and tobacco plants expressing reporter genes such as firefly luciferase (LUC) or β-glucuronidase (GUS) and/or GFP in under the regulation of *AtPT1* and *AtPT2* promoters. The reporter genes were specifically induced under Pi starvation in both species. Quantitative analysis of LUC activity in AtPT2:LUC plants showed a rapid induction during Pi starvation and suppression upon resupply of Pi. The temporal and concentration mediated expression of reporter genes driven by *AtPT2* promoter is identical to that of the native gene. Relatively high level of expression of LUC and GUS was observed in root tips of AtPT2-LUC/GUS plants. In contrast the expression of reporter genes was absent in the root tips of AtPT1-GUS/GFP plants. The reporter gene expression driven by *AtPT2* promoter was also observed in young flowers, peduncle and silique junctions of Pi starved plants. This data suggests that AtPT2 may also be involved in the *in planta* mobilization of the nutrient during Pi starvation. Histochemical analysis of GUS expression in Pi deficient roots showed a relatively stronger signal in epidermal and endodermal cell layers, and in root hairs. None of the tested hormones (auxin, ethylene, cytokinin) or abiotic stresses (salt, low and high temperature) significantly altered the expression of reporter genes.

391 FCP-like Phosphatase Gene Family Members (AtFLP) Differentially Regulate Arabidopsis thaliana Abiotic Stress Signaling, Growth and Development

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Two nonallelic Arabidopsis thaliana (ecotype C24) T-DNA insertion mutants, *flp1* and *flp3*, were identified based on altered stress regulation of *RD29A* promoter activity that was monitored using the luciferase reporter gene (*RD29A::LUC*) imaging system. Cold or osmotic stress signaling induces *RD29A* expression, which is an indicator of stress adaptation. Genetic linkage analysis and complementation data establish that the recessive *flp1* and *flp3* mutations are caused by T-DNA insertions in *AtFLP1* and *AtFLP3*. These genes encode two (of four) members of the phylogenetically conserved phosphatases that dephosphorylate the C-terminal domain (CTD) of RNA polymerase II (RNAP II); a process that inhibits transcript elongation. The *flp1* mutation causes *RD29A::LUC* hyperexpression in response to cold, ABA and NaCl treatment while the *flp3* mutation mediates hyper-responsiveness to ABA only. *flp1* plants accumulate biomass more rapidly and exhibit delayed flowering relative to wild type whereas *flp3* plants grow slowly and flower early compared to C24. Hence AtFLP1 and AtFLP3 are negative regulators of stress responsive gene transcription and are modulators of growth and development. This is the first evidence that plants use CTD phosphatases regulate transcription elongation and this is a focal control point of complex processes like plant stress responses and development. Apparently, AtFLPs have both unique and overlapping regulatory circuit functions, perhaps through differential regulation of distinct and common gene sets.

Research was supported, in part, by the NSF Plant Genome award DBI-9813360.

392 Profiling Cold-, Osmotic and NaCl-stress Regulated Gene Expression in Arabidopsis Using an Affymetrix GeneChip.

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Plants have evolved mechanisms that allow them to respond to changes in environmental conditions; these mechanisms often involve changes in gene expression. Once we know which genes and/or pathways are important to that response, we can use those sequences in transgenic approaches to develop crop plants that are more tolerant to abiotic stress. One of our goals is to create a database of *Arabidopsis* gene expression patterns delimiting interrelationships among stress-regulated genes identifying those that are uniquely regulated by one stress, and those that are regulated by multiple stresses. This database will help us define how a plant responds to stress and will likely identify candidates for use in transgenic analysis. We used the Affymetrix GeneChip to profile changes in gene-expression in response to low temperature, salt-stress and osmotic stress. The analysis of those results will be presented.

393 Effect of far-red irradiation on the development of the isolated meristem complexes and seedlings of *Arabidopsis thaliana*

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Pre-incubation of seeds of *Arabidopsis thaliana* in dim far-red light (730 nm, 210 mW/m^2) during 3 days caused development of plants with adventitious and lateral roots on B5 Gamborg medium during 15 days in white light (40 W/m^2). Isolated apical meristem complexes pre-incubated during 3 days in dim far-red light regenerated to give rise to plants with adventitious roots after 41 days of incubation on B5 medium. Control plants obtained after pre-incubation of seeds and isolated meristems in darkness developed only primary roots and did not produce the lateral and the adventitious roots. Incubation of seeds in white light during the supplementary irradiation by far-red light resulted in growth of plants with the adventitious and the lateral roots whilst the control seeds incubated only in white light produced plants only with the primary roots. Pre-incubation of seeds in dim far-red light caused the straightening of hypocotyls. During the incubation of seeds in white light or in white light with supplementary far-red irradiation development of seedlings only with curved hypocotyl was observed. Results of our experiments provide the evidence of the involvement of the phytochrome in development of seeds and isolated meristems of *Arabidopsis*.

394 The Arabidopsis SUMO pathway

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SUMO (**s**mall **u**biquitin-like **mo**difier) is a member of a growing family of ubiquitin-related proteins. Like ubiquitination, protein SUMOilation is a enzymatically catalyzed formation of a isopeptide bond between C-terminal glycine residue of SUMO and a lysine of a target protein. SUMO tagging has been implicated in nuclear transport, intranuclear localization, modulation of signal transduction cascades and stabilization of some regulatory proteins that are proteolysed by ubiquitine/26 S proteasome pathway.

The *Arabidopsis* SUMO family has 8 members that can be grouped in to three subfamilies which may modify a different subsets of target proteins. We have identified enzymes of the SUMO conjugation pathway in Arabidopsis: the heterodimeric SUMO activation enzyme (SAE1/SAE2) encoded by three genes (*AtSAE1-1*, *AtSAE1-2* and *AtSAE2*) and the SUMO conjugation enzyme (SCE1) encoded by a single gene. SUMOilation of a target protein is reversible because SUMO moiety can be hydrolyzed from the tagged protein by deSUMOilation enzymes (ULPs).

Analyses of expression of SUMO pathway genes, insertional mutants and plants overexpressing SUMO tags led to the conclusion that SUMO pathway is necessary for normal development and stress response.

395 Functional analysis of the 1Cys-Peroxiredoxin in Transgenic Tobacco Plants

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To date, two possible functions have been proposed for the plant 1Cys-peroxiredoxin, one as a dormancy regulator and one as an antioxidant. The transcript level of rice 1Cys-peroxiredoxin (R1C-Prx) rapidly decreased after imbibition of rice seeds, but the protein level was lasted for 15 days after imbibition. To investigate in vivo the real function of this protein, we generated transgenic tobacco plants constitutively expressing the R1C-Prx gene. The wild type and the transgenic R1C-Prx plants did not differ in their germination frequency, and both seeds exhibited more than 95% germination at 6days after imbibition. To measure the resistance of the transgenic R1C-Prx plants to radical stress, the whole wild type and transgenic R1C-Prx plants were infiltrated with 5mM H2O2 solution and analyzed the oxidative damage of the plants. Then, the wild type tobacco plants showed serious lesions up to 12th or 13th leaf from the top, whereas the transgenic R1C-Prx plants showed similar damage only up to the 4th or 5th. And, in general, the older leaves of both plants damaged more seriously than the younger ones. The resistance against oxidative stress was confirmed again by employing an OxiBlot reagent kit, which immunologically detects the carbonyl group on oxidized proteins. The DNP-hydrazone generated from the reaction of protein carbonyl groups with 2,4-dinitrophenyldrazine, can be detected by an antibody specific to the DNP moiety on the proteins. In this experiment, the protein carbonyl contents present in the 10th leaf from the bottom of the transgenic R1C-Prx plants treated with 5mM H2O2 were much less than those of wild type plants. From these results, it can be concluded that the in vivo function of 1Cys-Prx in plants may not be related to the maintenance of seed dormancy, but rather to protective activity against oxidative stress.

396 Overexpression of a Tonoplast H+-Pump Increases Biomass and Tolerance to Abiotic Stresses in Transgenic Plants.

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A major goal of agricultural research is improvement of crop yield. Abiotic stresses such as drought and progressive salination of soil threaten to compromise agriculture productivity in many regions. Plant vacuoles play a central role in cellular mechanisms of adaptation to these stresses. We have engineered Arabidopsis thaliana plants to overexpress the vacuolar pyrophosphatase H+ pump, AVP-1. Transgenic plants tolerate high salinity (250 mM), drought, and are larger than wild type plants. AVP-1 overexpression also increases solute accumulation and water retention. The shoot and root regeneration capability of leaf explants of the trasngenic plants is dramatically enhanced when compared to wild type. Data will be presented consistent with the hypothesis that an increased number of AVP-1 molecules enhances meristematic competence in transgenic plants.

397 Overexpression of enzymes involved in the biosynthesis of phytochelatin in *Arabidopsis thaliana* enhances arsenate and mercury resistance

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Phytochelatins (PCs), a class of heavy metal-inducible peptides, play a pivotal role in accumulation of and tolerance to thio-reactive heavy metal ions. PCs biosynthesis is catalyzed by three enzymes- g-glutamycysteine synthetase (g-ECS), glutathine sythetase (GS) and phytochelatin synthetase (PS). To test the phytoremediation potential of g-ECS, GS and PS in plants, we engineered *Arabidopsis thaliana*, to overexpress two bacterial genes, g-ECS and GS, one fission yeast PS gene (PBPS) and one *Arabidopsis* PS gene (AtPS). They were expressed under the control of *Arabidopsis* actin (ACT2), soybean rubisco small subunit (SRS1) and viral CaMV 35S promoters. Protein blot analysis revealed that g-ECS, GS and AtPS were overexpressed in transgenic *Arabidopsis*, but PBPS was not expressed in any of the lines analyzed. Heavy metal resistance studies demonstrated that g-ECS transgenic plants were highly resistant to arsenate (250-300 mM) and mercury (50-65 mM). Unlike the g-ECS transformants, GS expressing plants showed very little resistance to heavy metals. AtPS transgenic plants had intermediate level of tolerance to arsenate and mercury. None of these transgenic *Arabidopsis* lines were resistant to cadmium. Metabolic engineering of complex pathways in plants requires coordinate expression of the pathway related genes, and overexpression of single gene can play only limited role. Our future work, therefore, will examine the coordinate overexpression of g-ECS, GS and AtPS, either by classic genetic introgression or by IRES mediated polycistrons.γγγγγ??γγ

398 Characterization of a novel gene expressed in response to stress encoding a protein with a putative F-box.

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The F-box protein families define a class of molecule adaptors wich are component of the E3 ubiquitin-ligase complex named SCF (Skp1 CdC53 F-box protein).A diversity of cellular and regulatory functions in eucaryotic cells have revealed the importance of the regulated protein degradation process. The selectivity of the target protein, in many cases, is devoted to the carboxy terminal region of a F-motif protein. In *Arabidopsis* , several F-box proteins had been characterized (Tir1, Coi1, Ufo) however, a link between the stress response and the ubiquitinization process had been suggested by indirect evidence.In this study, we analized the pattern of expression of a gene wich encodes a putative F-box protein (Atb5). Osmotic, saline stress and mechanichal wonding induced accumulation of the corresponding mRNA. Interestingly, the orthologous gene in common bean display a similar pattern of mRNA accumulation in response to the same efectors.We constructed transgenic plants with the Atb5 cDNA in antisense and sense orientation. Sense plants shown phenotypes wich suggest sequestering of several SCF complex; poor root system, lost of apical dominance, curly caulin leaves, pigment accumulation in seedlings and alteration in flowers and siliques. The phenotypes in antisense plants and the putative interaction of this F-box protein with Skp1 will be discused.

399 Natural Variation in *Arabidopsis* Light Signaling

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Plants have a sophisticated set of photoreceptors and responses that allow morphogenic and physiological adjustments to their light environment. Since the optimal response is different in different environments, variation in light response may allow plants to adapt to different light environments. We are interested in finding the genes responsible for natural variation in light response, with one goal being to study the molecular basis of adaptation and microevolution.

Hypocotyl elongation is inhibited by light and is readily measured in the lab. We measured hypocotyl elongation in 140 *Arabidopsis* accessions in response to various light and hormone conditions and found up to three-fold variation between accessions. To determine which photoreceptor pathways might be responsible, we examined response to white, blue, red, and far-red light and compared the response patterns to those of known photoreceptor mutants. Some accessions have novel response patterns whereas others may have changes in known pathways. Using a candidate gene approach, we identified a change in *PhytochromeA* as being responsible for reduced sensitivity to far red light in Lm-2. We are currently pursuing biochemical characterization of this variant phytochrome.

We also have used the Ler/Cvi RIL set (Alonso-Blanco and Koornneef, 1998) to map QTLs responsible for variation in light response. An average of five QTLs per light condition examined have been identified (most loci affect response to more than one light condition). Some loci map to regions with no known photomorphogenic mutants, but one maps near *PhytochromeB (PhyB)*, known to be important for response to red and white light. We created a near-isogenic line (NIL) which confirmed that this region is important for light response, and preliminary results from association tests suggest that PhyB may indeed be the QTL.

Our results demonstrate that there is wide variation in the light response of natural populations of *Arabidopsis*. Both candidate gene and QTL mapping approaches are valuable for determining genes responsible for natural variation.

400 Regulation and Functions of Calmodulin-Related TCH2 and TCH3

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Calmodulin is a major calcium receptor in eukaryotic cells and functions to mediate responses to changes in levels of calcium acting as a second messenger. We have identified two calmodulin related genes, called *TCH2* and *TCH3*, in Arabidopsis that show strong upregulation of expression in response to a variety of stimuli, including touch, darkness, and temperature shocks. *TCH::GUS* expression and TCH3 protein accumulation correlate with sites of mechanical strain and cell expansion; these data indicate that the TCH2 and TCH3 functions may be required at these sites during plant growth and development and may be additionally necessary during responses to environmental stress.

TCH2 and TCH3 share 44% and 60% amino acid sequence identity with calmodulin and can bind calcium; it is likely, therefore, that the TCH proteins function to mediate responses to calcium fluctuations. Sequence divergence from calmodulin, however, indicates that the targets of TCH2 and TCH3 are likely to be distinct from those of calmodulin.

We are searching for proteins that interact with TCH2 and TCH3 in a calcium-dependent manner to identify potential targets. The three dimensional structure of TCH2 is being investigated. In addition, we are identifying plants with altered expression of and mutations in the *TCH2* and *TCH3* genes to elucidate the physiological functions of the proteins.

401 Characterization of a new *Arabidopsis* gene family encoding highly conserved hydrophobic proteins

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RCI2A and *RCI2B* are two *Arabidopsis* *R*are-*C*old-*I*nducible genes whose expression is also regulated during development and in response to dehydration, salt stress and ABA. They encode small (54 amino acids) highly hydrophobic proteins containing two potential transmembrane domains. Taking advantage of the completion of the *Arabidopsis* genome sequence we searched for genes having homology to *RCI2A* and *RCI2B* . We have found that *RCI2A* and *RCI2B* belong to a gene family composed by 8 genes. This work focuses on the organization, structure, expression, phylogeny and evolution of these genes. *RCI2s* are distributed among chromosomes 1, 2, 3 and 4 but members of the family were not found in chromosome 5. The deduced amino acid sequence showed that all proteins have the two potential transmembrane regions highly conserved, four of them showing an extra hydrophilic C-terminal domain of about 20 residues. Expression analysis indicated that the different genes are differentially regulated during *Arabidopsis* development and in response to abiotic stresses. A search in the data bases revealed that proteins showing high-sequence similarity to RCI2s are present not only in all kind of plants but also in very diferent living organisms ranging from bacteries to worms, suggesting a conserved and important role for them throughout evolution.

402 Analysis of the control mechanism for the level of the active oxygen species in *Arabidopsis*

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Stabilization of the level of the active oxygen species (AOS) is important for the survival of an organisms. In order to clarify the control system of plant AOS, super oxide radical (O_{2-}) scavenging activities were measured in the wild type (Col and L*er*), anthocyanin mutants (*tt3, ttg1*) and an ascorbic acid mutant (*vtc1*) of *Arabidopsis thaliana* by an ESR (electron spin resonance) method. Under ordinary growth conditions, *Arabidopsis* contains about 300-500 SOD units/g fresh weight radical scavenging activity. The ESR pattern indicates that most (40-50%) of this activity is due to ascorbic acid. To analyze the case under condition of oxidative stress, the synthesis of AOS was induced by gamma irradiation. The radical scavenging activity is increased about 10-fold following the accumulation of ascorbic acid and anthocyanin. These accumulations were suppressed by treatment with an antioxidant before irradiation and induced by treatment with a radical generating reagent. To confirm the activity of ascorbic acid and anthocyanin against AOS stress, we have examined the viability of the wild type and mutants (*tt2, tt3, tt5, ttg1, vtc1*) after gamma-irradiation. Only the ascorbic acid and anthocyanin-inducible plants had the ability to grow and flowered. Two ecotypes such as Col and L*er* show the difference of the contribution of ascorbic acid and anthocyanin. To identify the genetic locus of ascorbic acid and anthocyanin accumulation, we have used RI line. In L*er*, ascorbic acid accumulated twice the level in Col, and induction of anthocyanin was half that in Col. Now we are trying to define a genetic locus that control accumulation of ascorbic acid and anthocyanin with L*er* X Col recombinant inbred line.

403 Isolation and characterization of *Arabidopsis* mutants altered in cold-, salinity-, and ABA-induction of *rd29A* gene expression

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To dissect the signal transduction of drought, salinity, and cold stress responses, we isolated and characterized *Arabidopsis* mutants altered in their responses to these stresses. We introduced a chimeric gene construct consisting of the luciferase (*LUC*) under the control of the dry-, salinity-, cold-, and ABA-responsive *rd29A* promoter (*rd29A*::*LUC*) into *Arabidopsis* plants. Activation T-DNA tagged lines of *Arabidopsis* containing *rd29A*::*LUC* were obtained by transformation; mediated by *Agrobacterium* carrying the activation tagging vectors pPCVICEn4HPT or pSKI015. Some mutants altered in the regulation of the *rd29A*::*LUC* gene were identified. One of these mutants, #1986, showed constitutive induction of *rd29A*::*LUC* expression. Physiological, molecular, and genetic analysis of the mutants is in progress.

404 Elicitor and pathogen-dependent activation of a peroxidase promoter from *Arabidopsis* in transgenic reporter plants. A tool for the isolation of mutants showing aberant peroxidase expression

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The promoter of the *Arabidopsis* gene encoding peroxidase*CA (prxCA)* was fused to firefly luciferase as a reporter gene. Transgenic tobacco and Arabidopsis plants were generated and the expression of the reporter gene analysed *in-planta* using a photon-counting video system. The reporter gene cassette was locally activated in leaves of Arabidopsis and tobacco upon infection with avirulent *Pseudomonas syringae* and infiltration of culture medium of *Fusarium oxysporum*. The Arabidopsis reporter lines carrying the $prxC_A$-Luc constructs have been mutagenized and screened for mutants exhibiting constitutive high expression of the $prxC_A$ gene. Several mutants with high luciferase expression were isolated and are currently analysed in detail. Cell suspension cultures were initiated from transgenic tobacco reporter plants to study the signalling cascade leading to the activation of the reporter-gene construct. These cell lines responded to *Fusarium oxysporum* culture by reporter gene activation and this reponse required Ca2+-fluxes, oxidative burst and protein kinase (MAP-kinase) activity.

405 Analysis of blue-light signaling pathways using a *cry1 cry2 nph1 npl1* quadruple mutant.

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We have recently identified a new blue light receptor, npl1, and indicated a functional redundancy between nph1 and npl1 in phototropism and chloroplast relocation using the *nph1 npl1* double mutant. Although the *nph1 npl1* double mutant shows almost no phototropic response, we detected a slight phototropic curvature induced by other blue light receptor(s). This observation is one of examples showing the functional redundancy of photoreceptors in plant. To understand the relationship of the blue light signaling pathways, we made a *cry1 cry2 nph1 npl1* quadruple mutant and observed its phenotype on the blue light responses. Furthermore, we are trying the DNA microarray analysis of gene expression regulated by blue light using triple and quadruple mutants.

406 Interaction specificity of the two thioredoxin-h proteins with thioredoxin reductases in Chinese cabbage

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Even though three isotypes of thioredoxins, -f, -m and -h types, have been identified in plant cells, there are only a few researches on thioredoxin-h discovered at recent. In this study, two cDNAs encoding h-type of thioredoxin were isolated from a cDNA library of Chinese cabbage, and named here CTrx-h1 and 2. Deduced amino acid sequence of the CTrx-h proteins showed the highest sequence identity with those of arabidopsis thioredoxin-h2 (75.2%) and thioredoxin-h5 (46.6%) proteins, but they shared low sequence homology to other isotypes of plant thioredoxin-m and thioredoxin-f. Proteins encoded by the two CTrx-h genes, when expressed in E. coli, represented insulin reduction activities. However, whereas the recombinant CTrx-h1 was able to efficiently receive electrons from thioredoxin reductase, the CTrx-h2 could not accept electron from the protein, which might suggest that there was strong interaction specificity between the thioredoxin and thioredoxin reductase proteins. Genomic Southern blot analysis using the cDNA insert of CTrx-h1 revealed that the proteins consisted of a small multigene family in Chinese cabbage genome. On the contrary to CTrx-h1 that was widely distributed in most tissues of plant, the CTrx-h2 gene was predominantly expressed in flowers, but the expression was very low in other tissues. The result of Northern analysis suggests that the CTrx-h2 may have other function in flower development or differentiation, in addition to its defensive role.

407 Functional Analysis of *AREB* Genes in the Dehydration and ABA Specific Gene Expression of *rd29B* in *Arabidopsis thaliana*

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To understand the signal transduction pathways from the drought stress signal to gene expression, we have characterized *rd29B* gene from *Arabidopsis thaliana*. The induction of dehydration-responsive gene *rd29B* is mediated by abscisic acid (ABA). We have shown that ABA-responsive element binding proteins - AREB1 and AREB2 function as transcriptional activators in the ABA-inducible expression of the *rd29B* gene under dehydration condition. Meantime, we have isolated *Arabidopsis* genomic DNAs of AREB1 and AREB2. Analysis of the promoter region of these genes were carried out by fusing *β-glucuronidase* (*GUS*) reporter gene. An intron having 321-bp in the genomic DNA sequence of 5'upstream promoter region of *AREB1* was found. *AREB1* construct containing the 321-bp intron had a significantly higher GUS activity than that of control when the transgenic tobacco plants were exposed to dehydration. A similar high GUS activity was also observed in transgenic *Arabidopsis* seedlings with this construct while seedlings were exposed to dehydration, exogenous ABA and NaCl conditions. Histochemical analysis of *Arabidopsis* seedlings showed a high correlation with its GUS activity. Transgenic *Arabidopsis* seedlings having *AREB1* construct containing the 321-bp intron showed that GUS stained strongly in leaf, stem and root under dry, exogenous ABA and NaCl conditions. Deletion analysis towards the 5'upstream region of *AREB1* having 5'UTR 321-bp intron showed a decreasing trend in GUS activity as long as the deletion was imposed. Drastic decrease in GUS activity was observed with the deleted construct having 551-bp (-127- to +424-bp) which includes 321-bp 5'UTR intron. To identify the *cis* regulatory element involved in dehydration and ABA specific expression of *AREB1* gene, further deletion analysis both towards 5'and 3'region of -373- to +424-bp and 3'region of -127- to +424-bp are underway and will be discussed.

408 TOC1 (Timing of CAB expression 1): circadian regulation during reproductive growth

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The timing of reproductive growth in *Arabidopsis* is co-ordinated with the external environment by the action of a complex internal clock. The 'circadian clock' is regulated by the sun to follow the earth's 24hr cycles.

TOC1, a component of the 'circadian clock.' shows homology at the C-terminal region to CO (CONSTANS) a flowering time regulator, and at the N-terminal region to phospho-relay genes. TOC 1 is identical to AIP1 (ABI3 interacting protein 1), a protein identified via its interaction with the transcription factor ABI3 (ABA insensitive 3) in the yeast-2-hybrid system by Kurup *et al*. (2000). ABI3 is a major regulator of seed differentiation and maturation and also influences vegetative quiescence and bud dormancy.

If TOC1(AIP1) is functioning during seed development it could be a link between circadian control and transcription factors during embryogenesis. Fluorescent protein constructs of ABI3 and TOC1(AIP1) have been produced to investigate potential TOC1(AIP1)/ABI3 interactions within the nucleus. Antisense *TOC1(AIP1)* plants show altered flowering times under long and short-day conditions. Antisense toc1-1 plants have been cross-fertilised with mutant plants containing combinations of abi3-4 and co5abi3-4. These double and triple mutants will be used for phenotypic analysis.

409 Cloning *FRD3*: a novel integral membrane protein implicated in iron deficiency responses in Arabidopsis.

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In response to iron deficiency, all plants except the grasses induce Fe(III) chelate reductase activity, Fe(II) transport activity and proton release into the rhizosphere. Previously, we identified an Arabidopsis mutant, *frd3*, that constitutively expresses all three of these iron deficiency responses. Therefore, it is tempting to speculate that *FRD3* encodes a regulatory factor involved in sensing and/or responding to iron levels in Arabidopsis. The *FRD3* gene has been cloned; all three alleles of *frd3* have single base pair alterations in a single open reading frame. FRD3 is predicted to encode an integral membrane protein 526 amino acids long, to contain 10 to 12 transmembrane domains and to be localized to the plasma membrane. It is a member of a large family of membrane proteins, the best characterized of which is the *NorM* gene of *Vibrio parahaemolyticus* The NorM protein confers resistance to a variety of antibiotics and other toxic molecules, presumably through an energy-dependent efflux mechanism. Therefore, this protein family is known as the MATE (multi-drug and toxin efflux) family.

Experiments in pea have shown that there is a signal originating in the shoot that induces iron deficiency responses in the roots. Since *FRD3*, by RT-PCR, is expressed only in Arabidopsis roots, FRD3 may be the receptor for this shoot-derived signal. Alternately, FRD3 might be a transporter involved in transporting iron or a small signaling molecule into or out of root cells. Additional experiments are underway to characterize this novel protein and identify its role in iron nutrition.

410 A transcription factor from Arabidospsis acting downstream in the phosphate starvation signaling pathway

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Plants have evolved a number of adaptive responses to cope with growth in conditions of limited phosphate supply, involving biochemical, metabolic and developmental changes. We prepared an EMS-mutagenized M2 population of an Arabidopsis transgenic line harboring a reporter gene specifically responsive to Pi starvation (*AtIPS1::GUS*), and screened for mutants altered in Pi starvation regulation. One of the mutants, *Atphr1*1 (*phosphate starvation response 1*), displayed reduced response of *AtIPS1::GUS* to Pi starvation, and also had a broad range of Pi starvation responses impaired, including the responsiveness of various other Pi starvation-induced genes, and other metabolic responses, such as the increases in anthocyanin accumulation. *AtPHR1* was positionally cloned and shown to encode a transcription factor. A full size AtPHR1 protein fused to GFP was located in the nucleus independently of the Pi status. *AtPHR1* is expressed in Pi sufficient conditions and is only weakly responsive to Pi starvation. AtPHR1 was found to bind as a dimer to an imperfect palindromic sequence present in the promoter of Pi starvation responsive structural genes. Mutation of the AtPHR1 binding sequence in the promoter of *AtIPS1* greatly impaired its Pi starvation inducibility. These results indicate that this protein acts downstream in the Pi starvation signaling pathway.

411 *out of phase 1 (oop1)* plays roles in the regulation of

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A screen designed to identify mutants with altered circadian rhythmicity yielded *out of phase 1 (oop1),* which alters the phase of several rhythms including CO_2 assimilation, leaf movement, transcription rates of *LHCB* and mRNA levels of *CAT2* and *LHCB*. Because *oop1* is a novel allele of the red light photoreceptor phytochrome B (PHYB), we have more closely characterized the circadian phenotypes of other *phyB* alleles. To our surprise, the null allele *phyB-9* also displays altered circadian phasing in leaf movement and *LHCB* transcription. These findings raise the interesting possibility that, much like *Neurospora vivid* (Heintzein et al., Cell 104: 453, 2001) and *Synechococcus cikA* (Schmitz et al., Science 289: 765, 2000), *oop1* and other *phyB* alleles affect the phase of the clock through a mis-regulation of a clock component. In addition, although *oop1* and the recently described *phyB-28* (Krall and Reed, PNAS 97: 8169, 2000) are alleles of *phyB,* we present evidence that they affect blue light signaling mediated through CRY1 and/or PHYA. These two alleles accumulate truncated PHYB proteins that may prevent proper function of components of the CRY1 and/or PHYA transduction cascades. We will present molecular and biochemical approaches for the elucidation of the *oop1* phenotypes. This work was supported by grants from the National Science Foundation (MCB 9723482 and MCB 0091008).

412 ACTCAT: a *cis*-acting element involved in *Arabidopsis* proline dehydrogenase gene expression in response to hypoosmolarity and L-Pro

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Proline (Pro) is one of the most common compatible osmolytes in water-stressed plants. We obtained a cDNA clone for the proline dehydrogenase, ProDH, which is involved in the first step of the conversion of Pro to glutamic acid and a promoter region of the *ProDH* gene from *Arabidopsis thaliana*. We previously reported that the *ProDH* gene is upregulated by rehydration after 10-h dehydration, but downregulated by dehydration for 10-h in *Arabidopsis*. The *ProDH* gene is also induced by Pro.For further understanding of the expression of *ProDH*, we analyzed *cis*-acting elements involved in hypoosmolarity- and L-Pro-induced expression using deletion or mutated fragments of the *ProDH* promoter fused to the *LUC* (*luciferase*) or *GUS* (*β-glucuronidase*) reporter genes in transgenics. We found that a 70-bp *ProDH* promoter region contain *cis*-acting elements involved in hypoosmolarity- and L-Pro-induced expression of *ProDH*. Furthermore, base-substitution analysis revealed that the ACTCAT sequence in the 70-bp promoter region is important for the hypoosmolarity- and L-Pro-inducible expression of *ProDH*. Under dehydration condition, the 90-bp promoter region including the ACTCAT sequence did not respond to accumulated Pro. This suggests that this 90-bp region also contains negative regulatory elements for *ProDH* expression under water-stresses. We analyzed promoter sequences of other L-Pro inducible genes to find ACTCAT sequence in these promoters. We will discuss an important role of ACTCAT in hypoosmolarity- and L-Pro-inducible gene expression.

413 DET1, A Regulator of Arabidopsis Photomorphogenesis

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How do germinating seedlings maximize their response to their light environment? When Arabidopsis seedlings are grown in the light they exhibit short hypocotyls, open cotyledons and chloroplast development. In contrast, seedlings grown in the dark exhibit an etiolated phenotype, consisting of long hypocotyls, closed cotyledons, and lack of chloroplast development. De-etiolated-1 (det1) mutants display a light grown phenotype even in the dark, indicating DET1 is a key component in the light signaling pathway. DET1 has been cloned and found to encode a 62 kD novel nuclear protein of unknown biochemical activity. The results of a series of epitope-tagging experiments suggest that DET1 function requires nuclear localization and the formation of a 350 kD complex. Sequences in both the N- and C-termini of DET1 appear to be required for these properties. To determine the composition of the DET1 complex, myc-tagged DET1 was introduced into tobacco BY2 cells and the complex affinity-purified. The primary band co-purifying with DET1 is ~120 kD. By identifying this protein and determining its role in photomorphogenesis, we hope to gain insight into DET1's role in light response.

414 The *SKU5* gene plays an important role in controlling root tip axial rotation

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As wild type Arabidopsis root tips grow within a homogeneous medium, epidermal, cortical and endodermal cells longitudinally expand into cell files that exhibit minimal twisting about the axes of the roots. Root tips growing along a tilted impenetrable agar surface, on the other hand, periodically twist back and forth about their axes, forming roots that take on a sinusoidal wave pattern. Previously, we described the isolation and characterization of a mutant named *sku5* that was affected in a gene evolutionarily related to ascorbate oxidases and laccases. Unlike wild type roots that wave nearly straight down the agar surface of a plate, *sku5* roots skew and loop strongly toward the left as they wave. *sku5* roots also grow to be slightly shorter than normal and have slower bending kinetics when gravistimulated. Here, we report that the skewed root waving phenotype exhibited by *sku5* seedlings can be attributed to a tendency of the root tips to twist preferentially in a counterclockwise direction (left-handed twist) about their axes. This abnormal twisting appears to not require a directional stimulus since it occurs when roots are grown within a liquid medium as well as on an agar surface being clinorotated. Furthermore, *sku5* root tip twisting does not occur at a constant rate under those conditions, but is variable with no apparent pattern. In addition to these data, we will present data describing *sku5* root responses to exogenously applied hormones and drugs.

415 Effect of the phytohormones on the development of *Arabidopsis thaliana* (L.) Heynh. in culture of the meristem complexes *in vitro*

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The *in vitro* meristem model system has been established to study the effect of endogenous and environmental factors on plant development. The meristem complexes of *Arabidopsis thaliana* composed of apical meristem with 1-3 leaf or floral primordia has been isolated micro-surgically in sterile conditions. The regeneration of plants in meristem culture has been conducted on Gamborg B5 medium with different phytohormones composition. Phytohormone-free B5 medium does not support the regeneration process both in vegetative and in prefloral meristem cultures. Prefloral meristem complexes on media supplemented only with kinetin (0.5 mg/l) or with kinetin (0.5 mg/l) and NAA (0.02 mg/l) mainly continue to produce vegetative organs. On medium contained only NAA (0.02 mg/l), prefloral meristems continue to produce the flower buds. Thus, our data shows that kinetin affects the reversion of isolated meristems from prefloral to vegetative state. We could assume that the cytokinins might play certain role in the vegetative development of *Arabidopsis thaliana*.

416 A genomic approach to dissect shade avoidance responses in Arabidopsis

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Arabidopsis is a typical “shade avoiding” plant. For example, when grown in far-red-rich light, which mimics shading by neighboring plants, Arabidopsis displays a reduction of cotyledon and leaf expansion and an increased elongation of hypocotyl and petioles. At the molecular level, it is known that phytocrome B, D and E function in the regulation of shade avoidance responses. The phytocrome signal transduction pathways by which FR-rich light perception is coupled to the changes in gene expression underlying the developmental responses are poorly understood. However, genes encoding HD-Zip transcription factors, *ATHB-2* and *–4*, specifically induced by FR-rich light, have been identified; ectopic expression of one of them (ATHB-2) is sufficient to induce a constitutive shade avoidance response. Several recent findings indicated that auxin and auxin transport system are also important components of the elongation process induced by shade, and lead to a model for Arabidopsis shade-induced responses. In order to identify the molecular components involved in the shade avoidance response, we took advantage from the microarray technology. We used microarrays containing 11521 Arabidopsis ESTs (AFGC Microarray Facility, Michigan State University) to identify genes that are differentially expressed in plants treated with FR-rich light. At least 300 ESTs showed a difference of expression greater than two-fold in two independent experiments. As a first step to characterize these genes, we searched for those related to auxin. In particular, among the ESTs induced by FR-rich light, we found two ESTs related to two different auxin-repressed genes from *Fragaria ananassa* and from *Prunus armeniaca*, respectively, and four ESTs corresponding to auxin-induced genes. These belong to the known families of early auxin responsive genes, IAA and SAUR. Furthermore, we identified an EST related to an auxin-induced gene from *Vigna radiata* strongly repressed in plants exposed to FR-rich light. Expression studies to further characterize the auxin and the light regulation of these genes are in progress.

417 Promoter analysis of erd1: A ClpA-homologous-gene, form Arabidopsis up-regulated in response to dark-induced senescence and dehydration stress.

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The *erd1* gene encodes a 97kDa protein with sequence homology to the regulatory subunit of an ATP-dependent Clp protease; and is thought to function in protein degradation in chloroplasts. In *Arabidopsis* accumulation of *erd1* mRNA was found to be strongly induced by dehydration and salinity stress, plus natural and dark-induced senescence. In each case mRNA accumulation was found to occur independently of ABA biosynthesis. Analysis of the *erd1* promoter was performed by fusing a number of discrete portions of the promoter to the coding region of the LUC (Luciferase) reporter gene. Luciferase activity was subsequently measured in transgenic plants after exposure to dehydration or dark conditions. These experiments revealed that a 69bp region of the *erd1* promoter may contain *cis*-acting elements involved in the senescence-induced expression of this gene. This 69bp region contains an ABRE (ABA responsive element)-like sequence (ACGTG) and a second ACGT sequence. Data from constructs containing base substitutions in each of these elements revealed that they may be both involved in *erd1* gene expression during senescence. Furthermore, experiments were performed in order to asses the activity of this 69bp sequence in response to other senescence-inducing conditions.

418 Arabidopsis Mutants with Altered Expression of *AtGSTF2*

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Glutathione S-transferases (GSTs) are enzymes involved in detoxification of xenobiotics and cytotoxic products formed during oxidative stress. Some GSTs from crop species have been well characterized because of their ability to detoxify herbicides, but little is known about the role GSTs play in plants under normal conditions, or their function in plants under oxidative stress. To understand the signal transduction pathways of oxidative stress-responsive genes, the regulation of one specific GST gene in Arabidopsis, *AtGSTF2*, is being used as a model. Expression of *AtGSTF2* mRNA and protein are induced by a wide variety of stimuli including hormones, herbicides, antioxidants, and prooxidants such as copper and hydrogen peroxide. The *AtGSTF2* promoter was fused to the GUS reporter gene to visualize activation of *AtGSTF2* expression. Mutants with aberrant expression of the AtGSTF2-GUS reporter were selected. Two classes of mutants were identified with altered responses to copper, those with high induction of *AtGSTF2* (*hig*), and those with low induction of *AtGSTF2* (*lig*). A third class of mutants has constitutive expression of *AtGSTF2* (*ceg*) in the absence of copper. Five *hig*, four *lig*, and two *ceg* mutants have been verified in the M3 generation. In response to copper treatment, *hig* mutants accumulate higher levels of *AtGSTF2* mRNA than wild type, while *lig* mutants accumulate lower levels. *Ceg* mutants have constitutively elevated levels of *AtGSTF2* mRNA. Each mutation is inherited as a single, recessive locus and complementation tests indicate that two *hig* mutants are allelic while the remaining mutants fall into separate complementation groups. Mapping of the *hig*, *lig*, and *ceg* mutations has been initiated using CAPS markers. *HIG5* has been mapped to chromosome 5 within 3 centimorgans of marker m555, and experiments are underway in order to identify *HIG5*. Information obtained from studying these mutants will not only reveal crucial aspects of the transduction pathway specific to *AtGSTF2*, but will also serve as a model for understanding the signal transduction pathways of other stress-responsive genes.

419 Oxidative Stress Tolerant Lines of Arabidopsis Identified by a Functional Genetic Screen

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A genetic screen has been developed to identify *Arabidopsis thaliana* lines that show enhanced tolerance to oxidative stress. The screen is based on the extent of seedling root growth under oxidative stress conditions. This provides a simple visible phenotype that also enables the recovery of modest oxidative stress tolerant phenotypes. Seven putative mutants have been identified from an activation-tagged mutant population that show tolerance to the conditions induced by 3-amino-1,2,4-triazole, and buthionine *S,R*-sulfoximine, inhibitors of catalase and glutathione synthetase, respectively. Lines showing clear and heritable long-root phenotypes in the primary screen were subjected to a secondary screen which consisted of determining total antioxidant and total phenolic levels in leaves from leaves of unstressed seedlings and performing additional tests for tolerance to oxidative stress conditions brought about by hydrogen peroxide and methyl viologen. One line identified, *oxt21*, shows striking resistance to the oxidative stress conditions employed in the screen and displays cross tolerance expressed as improved growth and survival under various conditions that cause oxidative stress. Genetic characterization of the *oxt21* mutant line has indicated that the phenotype is due to a single, dominant mutation. However, molecular characterization has revealed that *oxt21* is not caused by the activation-tagging T-DNA. Progress in gene cloning and further physiological characterization of the *oxt21* line will be presented.

420 Salt stress induces anatomical changes in ovules and embryos, ultimately resulting in seed abortion

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Plant stress dramatically reduces Arabidopsis fecundity. This species is particularly sensitive to drought or heat: mild or transitory stress can lead to the abortion of all of the developing ovules and embryos on a plant. We developed a system to examine the effects of transient salt stress on seed development—plants were grown in hydroponic media, permitting rapid change in nutrient solutions at different water potentials. Plants were stressed for 8 hours using a nutrient solution that was supplemented with 150 mM NaCl and then returned to standard growth conditions. Stressed plants exhibited rapid anatomical changes in the embryo sac and embryo axis. Twelve hours after the application of stress, we observed high amounts of callose deposition in the walls of integument and chalazal cells. The cells and organelles that are normally found within the embryo sac degenerated extensively 12 hours after the salt stress. Anomalous bodies often appeared within the embryo sac while it was degenerating. The integument cells showed an increase in cell wall thickness and a fraction of them died, as evidenced by cellular debris in the periphery of these cells. One possible interpretation of these data is that salt-stress induces programmed cell death of ovules. In contrast, the phenomenon of embryo abortion appears quite different. Callose initially accumulates around the embryo and the proximal portion of the endothelium. This is followed by a cessation of cell division within the embryo and endosperm nuclei. This retardation of growth appears sufficient to cause the abortion of embryos.

421 Analyses of transient activation of phospholipase C that function in hyperosmotic stress signal transduction cascade in Arabidopsis cell culture

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In order to understand the roles of the phosphoinositide (PI)-turnover in signal transduction in hyperosmotic stress, we analyzed transient activation of PI-specific phospholipase C (PI-PLC), a key enzyme in PI-turnover, in response to various osmotic stress in Arabidopsis T87 cultured cell.

In T87 cells, we analyzed changes in inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) content in response to hyperosmotic shock or salinity. We detected a rapid and transient increase in the level of Ins(1,4,5)P_3 within 30 s in response to hyperosmotic stresses caused by mannitol or NaCl. However, no transient increase was detected in cells treated with ABA. The Ins(1,4,5)P_3 increase in response to hyperosmotic stress was inhibited by PI-PLC inhibitors, neomycin and U73122, suggesting that the transient Ins(1,4,5)P_3 production was mainly due to the activation of PI-PLC. To elucidate a role of PI-PLC in hyperosmotic-stress signal transduction pathways, we analyzed the induction of drought-inducible genes in T87 cells treated with the PI-PLC inhibitors. We found that the induction of drought-inducible genes, such as *rd29A* (*lti78*/*cor78*) and *rd17* (*cor47*) that are controlled by the DRE/CRT *cis*-acting element, was partially suppressed by neomycin or U73122 (Takahashi et al. (2001) *Plant Cell Physiol.* 42, 214-222). To show the involvement of PI-PLC in hyperosmotic stress signal transduction more directly, we analyzed the responses to hyperosmotic stress in transgenic T87 cells by manipulating the levels of PI-PLC gene. Based on the expression pattern of nine homologues of PI-PLC in various tissues in Arabidopsis plants or in T87 cells, AtPLC1s and AtPLC2 were suggested to be concerned in osmotic stress responses, and then introduced into T87 cells for transgenic analyses. In the transgenic T87 cells in which AtPLC1s or AtPLC2 was overexpressed, not only the Ins(1,4,5)P_3 increase but also the induction of rd29A in response to hyperosmotic stress were enhanced. These results also support an important role of PI-PLC in hyperosmotic stress signal transduction cascade.

422 Identification of phytochrome A-regulated transcriptional networks by microarray expression profiling in Arabidopsis

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The phytochrome family of sensory photoreceptors directs adaptational changes in gene expression in response to environmental light signals. Using oligonucleotide microarrays to measure expression profiles in wild-type and phytochrome A (phyA) null-mutant Arabidopsis seedlings, we have shown that 10% of the genes represented on the array are regulated by phyA in response to a continuous far-red light signal. Strikingly, over 40% of the genes responding to the signal within 1 hour are predicted to encode multiple classes of transcriptional regulators. Together with previous data, this observation suggests that phyA may regulate seedling photomorphogenesis by direct targeting of light signals to the promoters of a master-set of diverse transcriptional regulators, responsible in turn for orchestrating the expression of multiple downstream target genes in various branches of a phyA-regulated transcriptional network.

423 SPINDLY participates in the gibberellin signal transduction and photoperiod pathways

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SPINDLY (SPY) is a negative regulator of gibberellin signal transduction. Loss-of-function *spy* mutants exhibit phenotypes similar to those of wild type plants treated with GA, including early flowering. We used the yeast two-hybrid system to look for proteins that interact with SPY. GIGANTEA (GI) specifically interacted with the TPR domain and the full-length SPY protein, both in the yeast two-hybrid system and in vitro binding assays. GI is a novel nuclear protein that has been shown to be part of the photoperiod and the phyB signaling pathways. *gi* mutations delay flowering time under long-day conditions and cause a long hypocotyl phenotype under continuous red light. Phenotypic characterizations of a *spy-4*/*gi-2* double mutant revealed that *spy* is epistatic to *gi* with respect to both the flowering time and hypocotyl elongation phenotypes, indicating that SPY acts together with GI in regulating both developmental processes. Interestingly, *spy* plants were found to have a long hypocotyl under far-red light, suggesting a role for SPY in phyA signaling.

424 *CYP72B1*: A regulator of brassinosteroid levels and photomorphogenesis.

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Brassinosteroids are growth-promoting hormones that may be involved in modulating plant plasticity in response to changes in the environment. Previous studies of brassinosteroids have concentrated on either their metabolic or reception pathways. We have recently identified a novel gene affecting brassinosteroid responses in plants, *CYP72B1*, likely to be involved in brassinosteroid inactivation rather than biosynthesis or perception. We hypothesize that CYP72B1 is a brassinosteroid hydroxylase catabolizing the most active form of the hormone, brassinolide, into an inactive form, 26-hydroxybrassinolide. By regulating the active levels of brassinolide, CYP72B1 may be acting as a modulator of brassinosteroid responses during plant development. Analysis of the *bas1-D* mutant (*phyB-4* activation tagged suppressor 1- dominant), caused by the amplified expression of *CYP72B1*, and transgenic antisense lines with reduced *CYP72B1* expression, led to the hypothesis that this gene regulates both brassinosteroid levels and photomorphogenic responses in *Arabidopsis* (Neff et al., 1999). Analysis of a T-DNA knockout mutation in *CYP72B1* confirms the role that this gene plays in seedling photomorphogenesis. Analysis of this null mutant also reveals a role for *CYP72B1* in influencing floral induction in response to day length. Transgenic tobacco over-expressing *CYP72B1* are drought tolerant. Physiological analysis of these tobacco plants suggests that abscisic acid hyper-responsivity may be contributing to this drought-tolerant phenotype. Together, these results suggest that CYP72B1 may be regulating active levels of brassinosteroids as modulator of plant development in response to changing environmental conditions such as water availability and light.

Neff MM, Nguyen SM, Malancharuvil EJ, Fujioka S, Noguchi T, Seto H, Tsubuki M, Honda T, Takatsuto S, Yoshida S and Chory J (1999) *BAS1*: A gene regulating brassinosteroid levels and light responsiveness in *Arabidopsis*. Proc. Natl. Acad. Sci. 96 15316-15323.

425 Analysis of the dominant-negative ATHK1 in *Arabidopsis*

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To analyze roles of two-component systems in plant signal transduction, we have so far cloned a cDNA encoding a hybrid-type histidine kinase ATHK1 from *Arabidopsis*. We have previously demonstrated that ATHK1 has a potential ability to act as an osmosensor by analyzing both the sensing (input) and catalytic (output) activities with yeast osmosensing-defective mutants.

In order to examine the function of ATHK1 in planta, we attempted to generate *Arabidopsis* plants transformed with mutated ATHK1 cDNAs. We initially found that ATHK1 forms a homodimer through each cytoplasmic region by yeast two-hybrid interaction analysis. We then constructed a cDNA libraly of the mutated ATHK1 using PCR-based random mutagenesis and co-transformed a yeast SLN1 deletion mutant with a wild-type ATHK1 cDNA. We screened dominant-negative ATHK1 mutants that inhibited the activity of the wild-type ATHK1, which in turn suppresses the yeast SLN1 deletion mutant, and isolated six candidates (ATHK1-1 to 6). Sequence analysis revealed that ATHK1-2 has an N-terminal deletion and ATHK1-6 has a nucleotide substitution at a putative ATP binding site. We are currently analyzing transgenic *Arabidopsis* plants overexpressing the dominant-negative ATHK1 cDNAs.

426 Auxin and growth dynamics in the root meristem of arabidopsis in response to water deficit

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We study the regulation of growth rate by cell production and expansion in the *Arabidopsis thaliana* primary root exposed to water deficit. Spatial profiles of cell expansion and division rates are obtained through kinematic analysis to elucidate the role of these processes in stimulating growth under moderate deficit and maintaining growth under severe stress. Velocity profiles were obtained with the use of a new image analysis program that estimates instantaneous velocities at high spatial resolution. Sharp transitions in cell expansion and cell production are revealed and comparisons of the different treatments show the local dynamics of expansion and division. Growth under severe stress is accomplished primarily through the maintenance of cell expansion, as cell production is decreased by 50% or more. During moderate stress, an increase in both cell production and elongation underlies the enhanced growth. Cell production is not always increased and is not essential as the percent growth increase is the same when cell division is inhibited. Moreover, cell elongation while always increased in wild type plants also is not essential, because the auxin mutant *aux1-7* enhances growth to the same extent as wild type by increasing cell flux only. To explain the stimulated elongation, our results are consistent with moderate stress inhibiting auxin polar transport or lowering auxin sensitivity allowing for increased cell growth. First, cell elongation in roots under moderate stress is less sensitive not only to exogenous auxin, but also to treatment with the polar transport inhibitor, NPA. Second, auxin responsiveness as assessed by GUS expression driven by the BA3 promoter is reduced near the zone of rapid elongation. Third, cell expansion is not stimulated by moderate water deficit in certain mutants that disrupt auxin responsiveness or polar transport, namely *axr1-12*, *axr3-1*, *aux1-7* and *rcn1*. These results establish a role for auxin in the coordinated regulation of cell production and cell expansion in the root meristem.

427 The Arabidopsis *LOS5/ABA3* Locus Encodes A Molybdenum Cofactor Sulfurase and Modulates Cold and Osmotic Stress Responsive Gene Expression

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To understand low temperature and osmotic stress signaling in plants, we isolated and characterized two allelic Arabidopsis mutants, *los5-1* and *los5-2*, which are impaired in gene induction by cold and osmotic stresses. Expression of *RD29A-LUC* (firefly luciferase reporter gene under control of the stress responsive *RD29A* promoter) in response to cold and salt/drought is reduced in the *los5* mutants but the response to ABA remains unaltered. RNA blot analysis indicates that the *los5* mutation reduces the induction of several stress-responsive genes by cold and severely diminishes or even completely blocks their induction by osmotic stresses. Despite its dramatic impact on these stress-responsive genes, the *los5* mutation does not affect the expression of upstream CBF/DREB regulatory genes. *los5* mutant plants are compromised in their tolerance to freezing, salt or drought stress. They are also ABA-deficient, as indicated by increased transpirational water loss and reduced accumulation of ABA under drought stress. A comparison with another ABA deficient mutant *aba1* reveals that the impaired low temperature gene regulation is specific to the *los5* mutation. Genetic tests suggest that *los5* is allelic to *aba3*. Map-based cloning reveals that *LOS5/ABA3* encodes a molybdenum cofactor (MoCo) sulfurase. MoCo sulfurase catalyzes the generation of sulfurylated form of MoCo, a cofactor required by aldehyde oxidase that functions in the last step of ABA biosynthesis in plants. The *LOS5/ABA3* gene is expressed ubiquitously in different plant parts and the expression level increases in response to drought, salt, or ABA treatment. Our results show that LOS5/ABA3 is a key regulator of ABA biosynthesis, stress-responsive gene expression and stress tolerance. The results also suggest that 'ABA-independent' signaling may not be completely independent of ABA, and its operation may require ABA-dependent factor(s).

428 Characterization of Chloroplast Clp proteins in *Arabidopsis thaliana*

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Although the study of molecular chaperones and proteases is now one of the most exciting and developing fields of research today, our understanding of such systems in chloroplasts of higher plants remains rudimentary. The protein environment in chloroplasts is complex and dynamic, with many processes requiring the action of one or more chaperones or proteases. The Clp/Hsp100 protein family is a newly discovered family of molecular chaperones that are present in almost all bacteria and eukaryotes. Besides being important chaperones, many Clp/Hsp100 also participate in protein degradation by associating with the proteolytic subunit ClpP to form the Clp protease complex. Higher plants have by far the greatest number and complexity of Clp proteins than any other group of organisms. 19 different Clp isomers in plants have been identified, most of these were located inside chloroplasts. Because of this diversity, we have adopted a functional genomics approach to characterise all Clp proteins in the model plant *Arabidopsis thalianat* . Our ongoing research strategy combines genetic, biochemical and molecular approaches. Central to these has been the preparation of antisense transgenic lines for each of the chloroplast Clp isomers. These trangenic lines will be analysed to determine the importance of each chloroplast Clp protein for plant growth and development.

This work was supported in part by grants from SJFR – Swedish Agricultural and Forestry Resource Council, Carl Tryggers Foundation for Scientific Research, STINT – Swedish Foundation for International Cooperation in Research and Higher Education and the Israel Science Foundation, BARD – US-Israel Binational Science Foundation.

429 *PDF2*, a gene encoding a homeodomain protein, regulates epidermal cell differentiation in *Arabidopsis*

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The shoot apex of angiosperms consists of clonally different cell layers from the outside to the inside and the outermost (L1) layer gives rise to the epidermis of the primary shoot body. Genes expressed in specific cell layers have confirmed the layered nature at the molecular level. Some of them have been shown to be expressed exclusively in the L1 layer and implicated in determining the features of the cell surface. Furthermore, the *Arabidopsis thaliana MERISTEM LAYER1* (*ATML1*) gene encoding an HD-GL2 class protein shows expression specific to the protoderm of developing embryos and the L1 cell layer of shoot apices. Similar expression patterns have also been reported for its homologous genes in maize. These observations suggest a regulatory role for the members of the HD-GL2 class in the L1 layer-specific gene expression and consequently in the epidermal cell differentiation. Our previous study has demonstrated that an 8-bp motif named the L1 box functions as a *cis*-regulatory element for L1-layer-specific expression of genes in the shoot system of *Arabidopsis*. Here we report the isolation and characterization of *PROTODERMAL FACTOR2* (*PDF2*), a new member of the HD-GL2 class homeobox genes, that is expressed exclusively in the L1 layer of shoot meristems and the protoderm of organ primordia. We show that the recombinant PDF2 protein can bind to the L1 box in mobility shift assays. While *PDF2* overexpression results in delayed flowering, reduced expression of *PDF2* causes morphological aberrations in sepal and petal epidermal cells. Furthermore, we demonstrate that *PDF2* expression is upregulated by the induction of *ATML1*. These data suggest that PDF2 acts downstream of ATML1 and plays a role in epidermal cell differentiation in floral organs possibly by regulating the expression of essential L1-specific proteins.

430 Role of auxin transport and signaling in pattern formation of the apical region of the *Arabidopsis* embryo

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In dicotyledonous plants, the apical region of the embryo exhibits bilateral symmetry as two cotyledon primordia are formed at the opposite sides of the presumptive shoot apical meristem. The *CUP-SHAPED COTYLEDON1* (*CUC1*) and *CUC2* genes are required for cotyledon separation and shoot meristem formation during embryogenesis. These genes start to express as a stripe in the medial region between the presumptive cotyledons, reflecting bilateral symmetry in the embryo. Mutations in the *PIN-FORMED1* (*PIN1*) gene, which is required for polar auxin transport, frequently cause partial fusion of cotyledons and alterations in their number and position, suggesting that *PIN1*-dependent auxin transport is required for establishment of bilateral symmetry and cotyledon separation. Mutations in the *MONOPTEROS* (*MP*) gene, which encodes a transcription factor that is thought to mediate auxin signaling, also cause partial fusion of cotyledons, suggesting that the involvement of *MP*-mediated auxin signaling in cotyledon separation. To study roles of auxin transport and signaling in the formation of the apical region of the embryo, we performed expression analysis of *CUC1* and *CUC2* in *pin1* and *mp* mutant embryos as well as double mutant analysis. The results suggest that auxin transport and signaling mediated by *PIN1* and *MP* are involved in separation of cotyledons and shoot meristem formation through regulating expression patterns of *CUC1* and *CUC2*.

431 Regulation of the shoot and root apical meristems by MEI2-like RNA binding proteins

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We are interested in how an unusual class of RNA binding proteins functions in plants to regulate morphogenesis. The founding member of this class, MEI2 from *Schizosaccharomyces pombe*, is required for the transition to meiotic development. Although MEI2 contains 2 splicing factor-like RNA recognition motifs (RRMs) and acts in the nucleus, the mechanism of its action is unclear. The protein is unusual in that its nuclear localisation depends on it binding a non-coding RNA, meiRNA, an interaction mediated by a third RRM which defines the MEI2-like class of proteins [1].

A role for MEI2-like proteins in plants was first suggested by an analysis of the *TERMINAL EAR 1* (*TE1*) gene of maize in which loss of function mutations lead to changes in the pattern of leaf initiation[2]. Consistent with this phenotype, *TE1* is normally expressed in semi-circular rings which bracket sites of leaf initiation, suggesting expression of the gene somehow prevents leaf intiation.

We have begun a comprehensive analysis of the 7 *MEI2*-like genes in Arabidopsis. Two of these, *TEL1* and *TEL2* (***T****ERMINAL* ***E****AR1*-)**l**ike, are more similar to *TE1* while 5 other genes, *AML1-5* (***A****rabidopsis* ***M****EI2*-**l**ike, more closely resemble *MEI2*. Expression studies of genes from both these subclasses reveal highly patterned expression in both the SAM as well as the RAM which begins in the globular stage embryo and persists throughout the life of the plant.

3 loss of function mutants that have been identified from pooled T-DNA lines show no obvious mutant phenotypes. However, double mutants reveal striking defects to both root and shoot development. Expression patterns of these genes in various mutant backgrounds suggest they may influence determination pathways in the SAM and RAM.

1) Yamashita et al. Cell 95:115-123
2) Veit et al. Nature 393: 166-168

432 Role of IAA Flux on Vascular Tissue Development in Arabidopsis leaves

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Inhibition of polar auxin transport affects leaf development and venation pattern (Mattsson et al., 1999, Development. 126:2979-2991). However, the site of IAA production and its route of transport have not been characterized. It was generally assumed that IAA was produced in the emerging leaf primordia and drained into the plant, causing the differentiation of the primary vein. This implies a basipetal differentiation of the midvein procambium, which is incongruent with its apparent acropetal differentiation from the shoot. To study the role of polar auxin transport in mediating vascular differentiation during leaf development, we used a monoclonal anti-IAA antibody to assay IAA distribution in Arabidopsis tissue sections. In parallel, we used transgenic plants harboring the IAA inducible promoters fused to the beta-glucuronidase (GUS) reporter gene to analyze IAA distribution. IAA signals were monitored in seedlings grown on media containing the auxin transport inhibitor, 1-N-naphthylphthalamic acid (NPA). We found that NPA inhibited IAA accumulation in shoot apices of germinating seedlings. However, IAA signals can be detected in older leaf primordia before the initiation of the primary and secondary veins. In addition, cotyledons from NPA-treated seedlings show higher IAA signals than the controls. These results suggest that the young shoot apical tissue, which consists of actively dividing cells do not produce or accumulate free IAA, rather IAA was transported to these tissues from other organs. Acropetal flow of IAA from the plant into the developing leaf primordia would explain the acropetal differentiation of the primary vein.

433 Role of the HD-ZIP III family of transcription factors in vascular development

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ATHB-8, -9, -14, -15 and IFL1/REV are members of a small homeodomain-leucine zipper family (HD-ZIP III) whose genes are characterised by expression in the vascular tissue. *ATHB-8*, a gene positively regulated by auxin, is considered an early marker of procambial cells and of wound cambial cells during vascular regeneration (Baima S et al, *Dev* 121: 4171, 1995), while *IFL1*/*REV* has been shown to be necessary for proper development of the vascular tissue as well as for lateral meristems initiation and normal organ formation (Talbert PB et al., *Dev* 121: 2723, 1995; Zhong R & Ye Z-H, *Plant Cell* 11: 2139, 1999; Ratcliffe OJ et al., *Plant Cell* 12: 315, 2000; Otsuga D et al., *Plant J* 25: 223, 2001). As a basis for mutant analysis, we investigated the tissues and events involved in the formation of vascular system in *Arabidopsis* inflorescence stem. Interestingly, we observed both a ring of vascular cambium, mainly producing xylem, and a phellogen, indicating that *Arabidopsis* undergoes a complete program of secondary growth also in the stem. Moreover, we found that the onset of cambial activity is temporally associated with the deposition of cellulose at the wall corners of the outermost layers of interfascicular parenchymatic cells. Upon lignification, these cells further differentiate into extraxylary fibres. Although microscopic analysis demonstrated that formation of the vascular system is not affected in two *En-1* transposon-tagged insertional *athb8* mutant, we found that overexpression of ATHB-8 in transgenic plants promotes vascular cell differentiation. During primary growth, procambial and interfascicular cells differentiated precociously into primary xylem and fibres, respectively. Moreover, the transition to secondary growth was anticipated. Stimulation of vascular meristems results in an increased production of xylem tissue and complex modifications of the growth of ATHB-8 transgenic plants.To extend our knowledge on the specific functions of HD-ZIP III genes, we have undertaken a reverse genetics approach to identify mutants corresponding to ATHB-9, -14 and -15. The characterization of vascular system differentiation in these insertional mutants is in progress.

434 Cyclophilin 40 regulates vegetative phase change in Arabidopsis

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During its development, a plant shoot progresses from a juvenile to an adult phase of vegetative growth, and from a reproductively incompetent to a reproductively competent state. In Arabidopsis, loss-of-function mutations in *SQUINT (SQN)* truncate the juvenile phase and have subtle effects on inflorescence morphology, but have no effect on flowering time or reproductive competence. *SQN* encodes the Arabidopsis homologue of Cyclophilin 40 (CyP40), a protein found in association with the Hsp90 chaperone complex in yeast, mammals, and plants. Several different approaches have been used to characterize the biochemical function of this protein. In a heterologous complementation assay, *SQN* rescues the slow growth phenotype of a mutation in *CPR7*, a *S. cerevisiae* Cyp40 gene. A yeast-two-hybrid screen was performed using SQN as a bait protein to isolate interacting proteins that may clarify the mechanism by which SQN affects vegetative phase change. Positive interactors are being characterized. Using stable transgenic lines overexpressing SQN-GUS, the protein was localized to both the cytoplasm and the nucleus. Finally, expression patterns of 8-day old Col and *sqn-1* seedlings were compared by hybridization to microarray chips representing about 8200 genes. Genes whose expression increased or decreased by 2-fold or more encode proteins which fall into several broad categories: cell wall proteins, transcriptional regulators, stress-response proteins and others.

435 Mutations affecting ovule morphology and integument identity

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Arabidopsis seeds are typically oblong, and dried seeds reflect the shape of the embryo within. Some aspects of seed morphogenesis are under maternal control, however, and the analysis of several mutants points to a role for the integuments in the control of early morphogenesis. We have begun a new project to study the development of seed integuments and have screened for seed shape mutants that also are defective in another aspect of integument identity—the ability to make mucilage. From a population of 9,000 plants, we identified 40 lines with round seed and completely or partially disrupted mucilage production. One mutation was allelic to *ettin*, and we subsequently found that previously-identified *ettin* alleles also had defects in integument layer formation and seed shape, but not mucilage production. A phenotypic analysis of the new integument mutants as well as preliminary complementation results will be presented.

436 Two bHLH genes are involved in Arabidopsis root epidermis cell specification

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In the Arabidopsis root epidermis two cell types, root hair cells and non-hair cells, arise in a distinct position-dependent manner. Several genes that influence this cell specification of root epidermal cells have been identified previously. Among those, mutations in the WD40 protein TTG have been shown to induce ectopic root hair formation, thus suggesting a role for this protein in controlling non-hair cell fate. Overexpression of the maize *R* gene (a bHLH transcription factor) can complement the *ttg* mutant phenotype and in wildtype seedlings is leading to an almost hairless root phenotype. These data suggest that an R-like bHLH protein exists in Arabidopsis that acts downstream of TTG in promoting non-hair cell specification. To identify possible candidates, we investigated the influence of two bHLH genes, recently shown to be involved in promoting trichome cell specification, on root epidermis development. Mutations in *GLABRA3 (GL3)* cause ectopic root hairs to form in a portion of the primary root. A more extreme ectopic root hair phenotype was observed in a double mutant background consisting of *gl3* and *egl1 (enhancer of glabra3)*. Expression of an *EGL1* antisense construct in wildtype roots does not generate a significant phenotype thus suggesting a redundant function for these two proteins in specifying non-hair cell fate. Overexpression of either *GL3* or *EGL1* under the control of the 35S promoter results in a reduced number of root hairs. Furthermore, as has been predicted by the analysis of 35S::maize *R* lines, overexpression of *EGL1* is able to rescue the *ttg* mutant phenotype and leads to ectopic expression of *GLABRA2*, a homeobox gene promoting non-hair cell development downstream of *TTG*. These data confirm the longstanding assumption about an involvement of bHLH protein(s) in specifying non-hair cell fate in the Arabidopsis root epidermis.

437 The WOODY gene (WDY) is required for vascular patterning in Arabidopsis thaliana

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The developmental ontogeny of the vascular system (consisting of xylem, phloem and [pro]cambium) is poorly understood despite its central role in plant physiology. We are studying the genetic control of vascular patterning during root development in Arabidopsis. By serial sectioning we have recently determined the cell lineage relationships of the vascular tissue in the root meristem: xylem cell lineages are specified early, whereas phloem and procambium are established through a set of asymmetric cell divisions (Mähönen et al. Genes Dev 14: 2938). Subsequently, we have confirmed this anatomical model by analyzing the status of a set of marker lines from the Haseloff collection. In search of mutants defective in the vascular patterning of the root, we have identified a recessive, seedling lethal mutant, *woody* (*wdy*). *wdy* seedlings have a short root with only occasional lateral branches. Whereas the outer layers of the *wdy* roots have a normal radial pattern, the vascular system is abnormal. In the *wdy* vascular cylinder, xylem characteristically takes over a larger domain than in wild-type. This is associated with a lack of anatomically normal phloem and procambium in some regions of the root. Unlike in the *wooden leg* mutant with exclusive xylem development accompanying a reduced cell number (Scheres et al. Development 121: 53), the number of cells in the *wdy* vascular cylinder does not seem to be affected. This is suggestive for a different developmental basis for the altered vascular anatomy in the two mutants. To analyze this further, we have recently introduced various informative marker lines to the *wol* and *wdy* backgrounds. Progress in the developmental characterization and genetic mapping of the *wdy* mutation will be presented.

438 Interaction between *Asymmetric leaves1* and *KNOX* homeobox genes in Arabidopsis.

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The shoot apical meristem comprises undifferentiated and dividing stem cells that give rise to derivatives from which organ founder cells are recruited. The three dimensional architecture of lateral organs is specifed during founder cell recruitment. Both meristem maintenance and lateral organ form are processes defined, in part, by a cascade of negative regulatory interactions between homeobox transcription factors (*KNOX genes*) expressed in the shoot apical meristem and the myb domain transcription factor *Asymmetric leaves1* (*AS1*) expressed in lateral organ primordia. We are using a genetic approach to further define interactions between components in the pathway from shoot apical meristem to lateral organ development.

439 Analysis of CRINKLY4-like Receptor Kinase Genes in Arabidopsis

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The maize CR4 receptor-like kinase regulates cell differentiation in the endosperm and the shoot. Mutation in cr4 leads to defects in the leaf epidermis and aleurone layer. The *Arabidopsis* genome encodes 5 receptor-like proteins related to maize CR4. AtCR4 is believed to be the CR4 homologue with 60% amino acid identity and all the characteristic motifs of the maize CR4. Proteins encoded by the other 4 *CR*4-*RELATED* (*CRR*) genes lack the carboxyl domain and have lower similarity with the maize CR4. Northern blotting showed that *AtCR*4 is expressed in shoot apical meristems and young leaves, flowers and siliques. It is not detectable in mature leaves and roots. *CRR*1 and *CRR3* are also expressed because ESTs are present in database. A knockout of *AtCR*4 has been obtained by screening the Wisconsin collection of T-DNA insertion lines. Heterozygous plants show 50% seed set and we have not recovered a homozygote, suggesting that *AtCR*4 is required in the megagametophyte. We are pursuing strategies to study the function in sporophyte development.

440 *STRUBBELIG* and the control of meristem size and early organogenesis

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Each organ has a characteristic size and shape. How is it achieved? The control of patterns of cell division and change of cell shape are the main ways. However, little is known about the communication between cells that has to be part of this control in the shoot apical meristem and the emerging organ primordium.

Here we present our analysis of the *STRUBBELIG* (*SUB*) gene. The *sub* mutant plants show a range of pleiotropic defects. They exhibit altered ovule development and thus partial female sterility. In addition, the inflorescence meristem size is variable (smaller with fewer flowers and bigger with more flowers). Flowers and floral organs are either missing or show a reduction of growth. The shape of the epidermis and cortex cells of the inflorescence stem is irregular. It indicates a role of *SUB* in controlling meristem size and lateral organ initiation and outgrowth

We have cloned the gene by positional cloning. Northern data showed that *SUB* is ubiquitously expressed in the plant. *In situ* hybridization experiments revealed that *SUB* mRNA accumulates in the inflorescence and floral meristems, and emerging organ primordia. *SUB* encodes a putative leucine-rich repeat (LRR) transmembrane receptor-like kinase. These findings contribute to our understanding of signaling and cell communication mechanisms during the control of cell proliferation in a developing organ.

441 The Role of PID Kinase in Auxin Regulation

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The plant hormone auxin controls diverse mitogenic and morphogenic events during plant development. Auxin signal-transduction regulates endogenous patterning processes, including early steps in meristem partitioning and formation of the plant vascular system. Auxin also regulates tropic responses to external stimuli such as light and gravity. Genetic screens have identified genes involved in auxin transport, auxin regulated gene transcription, and targeted protein degradation as critical elements in auxin signaling. However, the mechanism by which these various components interact and are coordinately regulated is not yet known. We have cloned and characterized the *PINOID*(*PID*) gene of *Arabidopsis* which encodes a serine-threonine protein kinase. The pleiotropic *PID* loss-of-function and over-expression phenotypes resemble those of known auxin signaling and transport mutants, consistent with a specific role for this gene in auxin regulation. Furthermore, PID is the first kinase associated with auxin specific phenotypes. Therefore, PID offers a unique and powerful tool to explore the effect of protein phosphorylation on auxin-mediated processes. Analysis of the *PID* sequence indicates that PID is a member of a novel class of plant serine-threonine kinases. We are using genetic, molecular and biochemical strategies to initiate detailed dissection of the role of PID and its homologues in the regulation of auxin activity in *Arabidopsis*.

442 *CUC1* and *CUC2* promote adventitious shoot apical meristem formation on calli

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In dicot, the shoot apical meristem (SAM) is formed between two cotyledons during embryogenesis. Calli induced from various organs can form SAM, after induction of adventitious shoot formation. We have isolated *cuc* (*cup-shaped cotyledon*) mutant, which lacks an embryonic SAM and forms fused cup-shaped cotyledons. We have shown that this phenotype was caused by double mutations of two loci, *CUC1* and *CUC2*, and cloned both. Even in *cuc1* and *cuc2* single mutants, efficiency of adventitious shoot formation from calli was reduced compared to wild type. These results suggest that these genes positively regulate not only SAM formation in embryo but also adventitious shoot formation from calli. Here, we analyzed the role of the *CUC1* and *CUC2* genes in adventitious shoot formation. We made calli transformed with 35S::*CUC1* or 35S::*CUC2* and then, induced them to form adventitious shoot. They produced shoots much faster and more amount than the calli transformed with a control vector. This indicates that *CUC1* and *CUC2* promote adventitious shoot apical meristem formation on calli. During embryogenesis, *CUC1* and *CUC2* are suggested to induce SAM through activation of the *STM*. *stm* mutant does not form SAM in embryo. Moreover, *stm* mutant calli formed only leaves without SAM. When we transformed *stm* muitant calli with 35S::*CUC1* or 35S::*CUC2*, they produced very many leaves, but no SAM again. This also shows that *CUC1* and *CUC2* induced SAM through *STM* activation even in calli.

443 Regulation of Stomatal Neighbor Cell Polarity and Asymmetric Division in Arabidopsis

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Stomata are spaced apart from each other by at least one cell. The key mechanism that establishes this pattern in Arabidopsis is that the asymmetric division in cells adjacent to stomata or their precursors is oriented so that the new precursor, the satellite meristemoid, is placed away (Geisler et al., Pl. Cell 2000, 2075-86). Because the division in neighbor cells plays a major role in stomatal patterning, we analyzed parameters that regulate the competence of these cells to divide asymmetrically in wild type leaves. In addition, cytological events were studied that might predict which cells have been selected to divide asymmetrically. Neighbor cell polarity correlated positively with division competence. Depolarization and other temporal and morphological markers correlated with a loss of division competence. Many more cells were polarized than divided asymmetrically. Thus, neighbor cell polarity is a marker of division competence and does not predict a commitment to divide asymmetrically.

The *too many mouths* mutation randomizes the plane of asymmetric division in neighbor cells. However, *tmm* neighbor cells were more or less correctly polarized. This suggests that TMM regulates the selection of the division site but is not required for the establishment of a default polarity in neighbor cells. Finally, the regulation of division competence appears to be a significant factor affecting the distribution and density of stomata. Supported by grants from the National Science Foundation (Nos. IBN-9505687 and IBN-9904826).

444 Positional cloning and characterization of *ALF4*, a gene required for lateral root formation

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Lateral root formation is the primary way in which plants enlarge their root system. Lateral roots arise postembryonically from a subset of pericycle cells via the coordination of cell division, cell expansion and differentiation. Much work in recent years has shown that lateral root formation is regulated at a number of stages by environmental and development signals. Our lab has taken a molecular genetic approach to isolate genes important for lateral root formation. The *alf4-1* mutant was isolated because of its greatly reduced number of lateral roots. The mutation blocks lateral root development at or before initiation and the defect is not rescued by the addition of auxin, a promoter of lateral root initiation. In addition, the *alf4-1* mutant is male sterile and has reduced cell expansion in several organs including leaves and the hypocotyl.

To clone *ALF4*, the *alf4-1* mutation was mapped to a 2 cM region of chromosome 5. By walking towards *alf4-1* from linked markers, the mutation was delimited to a 32 kb genomic interval. Complementation of the mutant with wild-type DNA combined with direct sequence comparison of wild type and *alf4-1* genomic DNA was used to identify the *ALF4* gene. The mutation is a deletion that eliminates a splice site within the *ALF4* gene. We are currently investigating whether this mutation results in a null phenotype. Comparison of *ALF4* cDNA and genomic sequences revealed a gene structure composed of at least 13 exons although the precise 5' end of the transcript has not been determined. The *ALF4* gene, found only once in Arabidopsis, encodes a predicted protein with no obvious similarities to known proteins. However ESTs from wheat and soy bean share significant homology with *ALF4* suggesting that *ALF4* is found throughout higher plant species.

To examine the tissue-specific expression pattern of *ALF4*, transgenic plants have been made containing a β-glucuronidase reporter fused in-frame to the first exon of *ALF4*. Preliminary results indicate that *ALF4* expression is limited to areas within leaves, the embryo and the root elongation zone. This expression pattern taken together with the observed *alf4-1* mutant phenotype suggests a role for *ALF4* in controlling cell elongation and/or cell fate determination.

445 Activation of *BRACTS* Induces Ectopic Development of Bracts in *Arabidopsis thaliana*.

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The plant body is a dynamic structure whose architecture changes to adapt to environmental fluctuations. A major change in plant architecture occurs during the transition between the adult and vegetative phases in *Arabidopsis thaliana*. This transition is characterized by the complete inhibition of leaf development and the induction and transformation of the axillary shoot into the flower. We are characterizing an activation-tagged mutant line, termed *bracts-1d*, that in fact releases the development of bracts from inhibition and produces flowers each subtended by a leaf. Molecular characterization of the *bracts-1d* mutation shows that we have tagged a putative transcription factor with a single C2H2 zinc-finger domain. RT-PCR and *in situ* hybridization analyses indicate that the *BRACTS* mRNA accumulates within the inflorescence meristem, young floral organs, and the developing seeds. We are currently identifying a knock-out mutant line to establish a complete loss-of-function phenotype for the *BRACTS* gene.

446 Rescue of the *shootmeristemless (stm)* mutant phenotype by expression of *STM* mRNA in a subset of its normal domain: implications for nonautonomous action of the STM transcription factor

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The *SHOOTMERISTEMLESS (STM)* gene product is required for development of the Shoot Apical Meristem (SAM) and all of its derived products including leaves, stems, flowers, and seeds. The *stm-* phenotype is dramatic: plants germinate with root, hypocotyl, and cotyledons but no SAM. *STM* mRNA and protein are expressed throughout the developing SAM from early embryogenesis until senescence. The 3.5 KB genomic region upstream of the STM translation start codon drives expression of the GUS reporter in only the peripheral zone (PZ) of the SAM although *STM* mRNA and protein are normally found in both the peripheral zone and the central zone (CZ) of the SAM. Little is known about the relative roles of STM in the PZ versus the CZ. In order to understand this better, the *STM* cDNA was expressed from this "partial" (PZ only) promoter in *stm-11*/*stm-11* plants. These transformed *stm-11*/*stm-11* individuals were indistinguishable from the wild type. When rescued *stm-11*/*stm-11* plants were challenged by placing them in conditions requiring their SAM's to be more productive, they behaved similarly to challenged wild-type plants. Thus expression of *STM* in a subset of its normal domain yields complete rescue of our strongest *stm* hypomorph. In order to understand how this could happen, expression of *STM mRNA* and protein was analyzed in rescued homozygous *stm–11* embryos. Surprisingly, the STM protein was found at wild-type levels in both the CZ and the PZ. In contrast, the *STM* mRNA was significantly reduced in the CZ . This result is consistent with the STM protein being expressed in the PZ and moving into the CZ in rescued embryos. We are pursuing further experiments to investigate this possibility, as well as the role of such movement in the normal development of the SAM. In addition, we have done similar rescue experiments in which KNAT1, KNAT2, or an STM-GUS fusion was expressed from the partial STM promoter in *stm-11*/*stm-11* individuals. The results of these shed some light on the wild-type functions of KNAT1 and KNAT2 and give insight into the probable mechanism of rescue of the stm phenotype.

447 The transposon insertion in a putative homeobox gene disrupts many aspects of normal development in Arabidopsis

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The screen of gene-trap insertion lines identified a new hypothetical homeobox gene that encodes a transcription factor of 210 amino acids. The insertion knock-out alters plant cell differentiation, cell division, and overall development. The transposant is severely stunted with abnormal root and shoot development, as well as atypical leaf shape. Plants grown on MS salts medium do not flower, they continuously sprout malformed leaves and sometimes callus develops on the petiol and hypocotyl. When the transposants are grown on soil, they can perform the transition into reproductive growth, producing few flowers and curved siliques. Morphological analysis of the root revealed a defect in radial organization, with irregular cortical cell distribution and a disorganized vascular bundle. In addition, lateral roots are nodule-shaped and leaves are thick and elongated with finger-like shape. Taken together, all these data suggest this putative homeobox gene plays an important role of manipulating plant development in multiple facets.

448 Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14*

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Lateral root development is one of the postembryonic organogenesis that gives rise to most of underground parts of higher plants. Although it is known that auxin promotes lateral root formation, the molecular mechanisms of auxin-regulated lateral root formation is still unknown. We identified a novel Arabidopsis locus *SOLITARY-ROOT* (*SLR*) which is important for lateral root formation and the other root morphogenesis. A dominant *slr* mutant completely lacks lateral root and cannot be rescued by the addition of exogenous auxin. Analysis with cell cycle and cell differentiation markers (*CycB1;1* ::GUS and End199) indicated that the *slr* mutation specifically blocks the cell divisions of the pericycle in the early stages of lateral root initiation. In addition, the *slr* mutant is also defective in root hair formation and gravitropic responses in both roots and hypocotyls. Physiological analysis showed that the *slr* roots are specifically resistant to auxins. We found that the *slr* has a point mutation (P82S) in the conserved domain II of IAA14, a member of Aux/IAA proteins. The expression of the mutated *IAA14* cDNA under the *IAA14* promoter caused the *slr* phenotype in wild-type plants. Furthermore, we isolated an intragenic suppressor mutant of *slr* (*slrR-1*) which has a second point mutation in IAA14. These results show that *SLR* encodes IAA14. We observed that the mutated IAA14-GFP fusion protein is specifically localized in the nucleus and that a gain-of-function *slr*/*iaa14* mutation decreases the auxin-inducible BA-GUS expression in the root, strongly suggesting that IAA14 acts in the auxin-responsive transcription. Our results indicate that SLR/IAA14 is a key component in auxin-regulated root development, especially in lateral root formation.

449 *Arabidopsis TFL2* gene, encoding a novel polycomb protein, makes a complex with CO-like protein and represses *FT* expression during floral transition.

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tfl2 mutants were first isolated as an enhancer of *tfl1* mutant. However, we focused on their day-length independent early flowering phenotype, since the *tfl2* mutation does not affect TFL1 protein trafficking.

We have cloned *TFL2* gene based on T-DNA tagged *tfl2-3* allele. *TFL2*gene encodes chromo and chromo-shadow domains and this type of polycomb protein is unique in the whole *Arabidopsis* genome. *TFL2* is expressed ubiquitously in the shoot meristem (vegetative, inflorescence, and flower). These suggest that *TFL2* functions as a negative regulator during floral transition. In order to reveal in which flowering pathway *TFL2* is involved, we compared *FT, LFY*, and *SOC1* expression level in the *tfl2* mutant and wild-type background. *FT* expression is highly upregulated much earlier than floral transition but *LFY, SOC1* are not much affected. By using the yeast two-hybrid screening, we have cloned CO-like Zn-finger proteins as interacting molecule with TFL2 protein. Together, these results strongly suggest that *TFL2* acts a repressor against the *CO-FT* floral induction pathway. Presently, genetic and TFL2-CO protein interaction analyses are in progress.

450 Mutations in the *Arabidopsis* and tomato *Lateral suppressor* genes reveal a common control mechanism for lateral shoot formation in monopodial and sympodial plants

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Plant growth depends on the activity of meristematic cells at the tips of shoots and roots, the apical meristems. In seed plants, shoot branching is initiated by the formation of new meristems in the leaf axils, which subsequently establish new axes of growth. The patterns of axillary bud formation and the growth characteristics of side-shoots determine to a large extent the form of plants. Analyses of mutants in different plant species suggest that the initiation of axillary meristems and the establishment of the main shoot apical meristem during embryogenesis are distinct processes governed by different control mechanisms.

Tomato plants carrying the recessive *lateral suppressor* (*ls*) allele are characterized by several phenotypic abnormalities, among which the absence of side-shoots and the failure to develop petals are the most prominent ones. The *Ls* gene was isolated using a map-based cloning approach. Based on microsynteny studies we have subsequently also identified the *Ls*-orthologous gene from *Arabidopsis*. Isolation of mutants from a population of plants carrying the transposable element *En*/*Spm* allowed us to compare the phenotypes of mutants in the monopodial *Arabidopsis* versus the sympodial tomato. Similar to the *ls* mutant of tomato also the *Arabidopsis* mutant shows a clear defect in lateral shoot formation. These observations suggest that the Ls protein is part of a common control mechanism conserved between monopodial and sympodial plants.

The Lateral suppressor protein belongs to the GRAS family of putative transcription factors. Members of this family, e.g. SCR and GAI, control diverse steps in plant development. RNA in situ hybridization experiments and GUS stainings of transgenic plants containing Ls::GUS constructs show that *Ls* expression is restricted to specific cell groups. Signals were found in the axils of leaf and sepal primordia as well as in root tips. These experiments suggest that the *Ls* gene product is interpreting positional information directing the establishment of new meristems.

451 Regionalization of the *Arabidopsis* embryo

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The outcome of embryogenesis is the basic body plan of the seedling, that consists of the shoot meristem, cotyledons, hypocotyl, root and root meristem. Embryogenesis starts with the first, asymmetric division of the zygote, leading to two daughter cells that give rise to different parts of the embryo. We are interested in understanding how the primary body plan of the seedling is established. We have identified three *Arabidopsis* homeobox genes, *ZOX1-3*, the expression patterns of which reflect the regionalization of the early embryo. All three genes are expressed within the zygote. During the first asymmetric division their mRNAs become localised to either the apical or basal daughter cell. After three more rounds of cell divisions, the *ZOX* genes mark the three principal domains of the early embryo: the apical four cells that give rise to the shoot, the basal four cells that give rise to the root, and the extraembryonic suspensor.We hypothesize that the *ZOX* genes determine different regional identities in the early embryogenesis. Furthermore our results suggest that the zygote expresses determinants for different embryonic regions and that different cell fates arise by segregation of these determinants via asymmetric cell divisions. We have isolated mutations in all three *ZOX* genes and are currently analysing their phenotypes.

452 Overlapping and Unique Functions of the Auxin Response' transcription factors MP and NPH4

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Auxin response factors (ARFs) constitute a family of transcription factors that can bind to conserved promoter elements in auxin-regulated genes (1). For most ARFs, the biological functions are not known. Two ARFs, MONOPTEROS (MP, ARF5, 2) and NON PHOTOTROPIC HYPOCOTYL 4 (NPH4, ARF7, 3), display extensive sequence similiarity in DNA binding and protein interaction domains, but have been implicated in distinct developmental processes: MP in the formation of the embryo axis and in vascular development (4), NPH4 in auxin-mediated differential cell expansion (5). Here we show that MP and NPH4 can act redundantly in embryo and vascular development. Analysis of mp;nph4 double mutants reveals the potential of NPH4 to promote embryo axis formation and vascular development, suggesting redundant regulation of downstream genes. In the regulation of another set of target genes, however, activity of both MP and NPH4 is required, suggesting that in the corresponding transcriptional complexes the two proteins have non-redundant functions. Physical interaction in common complexes is consistent with strong interaction of both gene products in yeast two-hybrid assays. Finally, interactions of both proteins is also indicated by synergistic phenotypes and downstream expression profiles resulting from single and double overexpression of the two genes in transgenic plants.[1] Guilfoyle et al. (1998). Plant Phys. 118: 341-347. [2] Hardtke and Berleth (1998). EMBO J 17: 1405-1411.[3] Harper et al. (2000). Plant Cell 12: 757-770.[4] Przemeck et al. (1996). Planta 200: 229-237.[5] Stowe-Evans et al. (1998) Plant Physiology 118: 1265-75.

453 Characterization of *frill1*, an Arabidopsis floral mutant with serrated petals and sepals

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Organ development is results from appropriate control of cell division and cell expansion, but we know only a little about this control. Wide range of mutants is necessary for a better understanding of the genetic control in organ development. We have isolated and characterized a *frill1* (*frl1*) mutant that has serrated petals and sepals, while the other floral and vegetative organs are not affected in this mutant. Petals and sepals of the *frl1* are relatively broader than those of the wild type. Previous observations revealed that the number of petal epidermal cells in the distal region was decreased but their size was variable and relatively larger in the *frl1* petal, as compared with those in the wild-type petal. Nuclear size was also larger and variable in the *frl1* petal but not in the wild-type petal, suggesting abnormal endo-reduplication occurred in the distal region of *frl1* petal. However, no significant difference was found in the basal region. Observations of the early petal development revealed that the *frl1* phenotype became apparent at the floral stage 10. These results indicate that *FRL1* gene is required to attain normal cell division and expansion in latter half of the petal development. Now we are isolating the *FRL1* gene using a map-based approach. *FRL1* gene is mapped in the 105 kb region at the middle of the upper arm of chromosome 1. Complementation analysis is in progress.

454 GIBBERELIN AS A COMPONENT OF HOMEOBOX PATHWAYS IN ARABIDOPSIS

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The formation of the shoot body of higher plants relies on the continuous organogenic and self-renewal activities of the shoot apical meristem. The exact nature of the developmental pathways governing shoot development is still poorly understood. Current evidence suggests that the precise mode of expression of *knotted1*-like homeobox (KNOX) proteins play a central role both in meristem function and in acquisition of leaf identity. We are interested in whether plant growth regulators act in or in conjunction with the KNOX pathway to regulate shoot development. Previous work by other researchers has shown that ectopic KNOX expression results in drastically lower gibberellin (GA) levels in rice and tobacco. Using a transgenic Arabidopsis line harboring a dexamethasone inducible *KN1* construct, we have established that GA strongly suppresses the lobed leaf phenotypes resulting from inappropriate KNOX expression. Here we present genetic evidence that repression of gibberellin biosynthesis not only mediates effects of KNOX misexpression in leaves but is also likely to be a critical factor for meristem function in Arabidopsis.

455 SCARECROW function in the quiescent centre

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The maintenance of a functional meristem requires coordination between the loss of stem cells through differentiation and the replacement of these cells through division. Laser ablation studies have shown that in roots, stem cell maintenance by the quiescent centre (QC) may be one of the ways to ensure continuous meristem activity.

SCARECROW (*SCR*) is one of the earliest genes expressed in the QC, its expression being first detectable during embryogenesis in the hypophyseal cell. In the seedling root, *SCR* is expressed in endodermis, cortex-endodermis stem cells and in the QC[1]. *SCR* and its homolog *SHR* encode putative transcription factors, both essential for the asymmetric division of the cortex-endodermis stem cell to produce cortex and endodermis[2]. However, the role of *SCR* in the QC remains to be resolved.

One aspect of the *scr* mutant phenotype is cessation of main root growth within 10 days post germination, which is correlated with meristem differentiation and loss of stem cells. We observed that several independent QC expressed markers are absent in *scr* mutants, indicating that QC identity requires *SCR* gene activity. We questioned whether restoring SCR activity in the morphologically identified QC of *scr* mutant plants would also restore continued meristem activity. However, first cell autonomy of SCR action was determined using a CRE/lox based clonal analysis whereby gene activation is visualized by GFP expression. Ectopic *SCR* expression in the *scr* mutant background induced periclinal divisions in cells of the mutant single ground tissue layer only in those cells where gene activity was induced. Interestingly, periclinal divisions were observed at random positions in the mutant ground tissue layer indicating that all meristematic ground tissue cells are competent to divide upon SCR activity. Subsequent restoration of *SCR* expression using different promoters marking the mophological QC enabled continued meristem activity and maintenance of root growth in a *scr* mutant background. In addition, QC marker expression was restored.

These results suggest that SCR contributes to QC identity in a cell autonomous fashion, and that expression in the QC is sufficient for non-autonomous maintenance of stem cell and meristematic activity.

1. Wysocka-Diller et al. 2000, Development 127:595-603, 2. Helariutta et al. 2000, Cell 101:555-67.

456 Cell cycle regulation during early lateral root initiation

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Lateral root development is considered to consist of three major steps, pericycle activation, meristem establishment and root outgrowth. In Arabidopsis thaliana the pericycle cells are arrested in the G2/M phase of the cell cycle at the moment of the lateral root initiation. The first formative divisions are characteristically asymmetrical and anticlinally oriented. The resulting founder cells divide periclinally to form the two-cell-layer stage (Casimiro et al., 2001. Pl. Cell 13;843-852). To efficiently study the molecular and cytological events during the early stages of the pericycle activation, we developed a lateral root inducible system. A treatment with an auxin transport inhibitor (N-1-naphtylphtalamic acid) followed by exogenous application of auxin (NAA) were used to prevent the lateral root initiation and to activate the whole pericycle, respectively. The pericycle activation was followed in a time course every two hours from 4 to 12 hours after transfer from NPA containing media to NAA. During this time course, developmental similarities with the natural situation were observed in the pericycle cell layer. By using RT-PCR technique an early down regulation in transcript levels of KRP-1,-2 and -4 genes (former Cyclin dependent Kinase Inhibitors) were detected at 4 hours. This was followed by induction of B-type cyclins, CYCB1;1 and CYCB2;1 at 6 hours. In histochemical GUS-assays with CYCA2;1 and CYCB1;1 promoter-fusion lines the first asymmetric cell divisions were detected at 8 hours and at 10 hours the whole pericycle consisted of small, radially expanded cells that continued dividing periclinally leading to the two-cell-layer stage at 12 hours. We have thereby shown that this enhanced system can be utilized in analyzing the cell cycle progression during the course of lateral root induction by histological and molecular techniques. Currently cDNA-AFLP is used to identify genes the expression of which correlates with the very early events in lateral root formation. Characteristic classes of the transcripts, such as NPA negative/NAA positive and early and late induced genes will be further analyzed.
Arabidopsis thalianaKRPCYCB1;1CYCB2;1CYCA2;1 CYCB1;1 Casimiro et al., 2001. Pl. Cell 13;843-852

457 Identification and Expression of the *VASCULAR PREPATTERN* Gene of Arabidopsis

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We are using gene trap technology to identify new genes involved in leaf morphogenesis. Vascular patterning is a fundamental aspect of this process of which the early stages of control are poorly understood. We report here the identification and expression of *VASCULAR PREPATTERN* (*VPP*), a novel gene marking the early stages of vascular development. In the enhancer trap line, the Ds insertion lies 250 bp upstream of the *VPP* transcription start site and does not affect its transcription. GUS activity is detected in provascular cells from the earliest stages of primary midvein formation in leaf primordia and subsequently coincides with the early specification of the higher order veins. GUS activity is evident prior to the morphological changes associated with vascular cell differentiation and is not observed in mature veins. The root quiescent center cells are marked by GUS activity at all stages of root development, suggesting a possible role for *VPP* in maintaining a stable root apical meristem structure. In situ hybridization was used to confirm the *VPP* expression pattern in developing leaves and to examine *VPP* expression during embryogenesis. Expression is first detected throughout the 8-cell stage embryo, and is subsequently excluded from the protoderm from the 16-cell stage onwards. In later stage embryos *VPP* expression is associated with the early root quiescent center and forming cotyledon and root vasculature. While auxin is known to play an important role in patterning of both the root meristem and vasculature, *VPP* expression is not regulated by auxin. *VPP* likely acts prior to the action of auxin and may play a role in establishing quiescent center and provascular cell identity. Functional characterization of *VPP* is now underway.

458 The Regulation of AGAMOUS

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The transcriptional regulation of *AGAMOUS (AG)*, a floral homeotic gene responsible for the development of stamens and carpels, is coordinated by enhancers located in the 3 kb intron. One of the enhancers contains two binding sites for the *AG* activator LEAFY (LFY) (Busch et al., Science 285:585; 1999) as well as several other putative transcription factor binding sites. We have begun an extensive analysis of these sequences using phylogenetic footprinting, reporter gene studies and rescue experiments. Among the putative binding sites for other transcription factors is a MADS-domain consensus binding site (CArG) that is highly conserved among 27 putative Brassicaceae *AG* orthologs. Furthermore, two consensus binding sites for HAP heterotrimeric complexes (CCAAT-boxes) are also well conserved, even among more distantly related species such as snapdragon and tomato. Mutations in the CArG box resulted in ectopic *AG::GUS* expression in the shoot apical meristem (SAM), suggesting that this site mediates repression of AG in the shoot. The MADS domain protein FRUITFULL is likely to act via the CArG box since this *AG* enhancer element is ectopically activated in *FUL:VP16* plants (collaboration with S. Sato, C. Ferrandiz & M. Yanofsky, UCSD). Deletions in the CCAAT-boxes led to a decrease in late *AG::GUS* expression in stage 9 carpels; these sites are therefore likely to be important in maintaining later levels of *AG* expression. Finally, we have determined to what degree the 3' enhancer containing these sites contributes to normal *AG* function by linking wild-type enhancers to a minimum promoter and *AG* cDNA and introducing them into *ag-2* null mutants. We found that the 3' enhancer, which is transiently active in the center of young flowers, could partially restore carpel features, such as style and stigmatic papillae, but not floral determinacy or stamen identity.

This work has been supported by NIH Training Grant GM07240-24 (R.H.), and by a grant from DOE (D.W.).

459 Cloning of an Arabidopsis Patatin-Like Gene, STURDY, by Activation T-DNA Tagging

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Cloning of an Arabidopsis Patatin-Like Gene, STURDY, by Activation T-DNA Tagging Shihshieh Huang 1, R. Eric Cerny 2, Deepti Bhat 3, and Sherri M. Brown4 ABSTRACT Activation T-DNA tagging can generate dominant gain-of-function mutants by overexpression of a particular endogenous gene. We identified an activation tagged mutant, sturdy, exhibiting a stiff inflorescence stem, thicker leaves, shorter siliques, larger seeds, round shaped flowers and delayed growth. Most importantly, unlike its wild type counterpart, this mutant is less prone to lodging. Cloning of STURDY revealed that in sturdy, there is an open reading frame containing a single intron encoding a patatin-like homolog. The T-DNA is inserted into the 3' region of the second exon. The mutant phenotype was shown to be the result of overexpression of STURDY by mRNA analysis and transgenic studies. Preliminary histological studies have revealed an increase in cell number in the inflorescence stem of mutant plants, however, additional studies are needed to better understand the overexpression phenotype.

460 The Arabidopsis *REF8* gene encodes the 3-hydroxylase of phenylpropanoid metabolism.

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The activity of *p*-coumarate 3-hydroxylase (C3H) is essential for the biosynthesis of lignin in plants, and yet no conditions suitable for the unambiguous assay of the enzyme are known. As a result, all attempts to purify the protein and clone its corresponding gene have failed. By screening for plants that fail to accumulate soluble fluorescent phenylpropanoid secondary metabolites, we have identified a number of Arabidopsis mutants that display a *reduced epidermal fluorescence* (*ref*) phenotype. Using radiotracer feeding experiments, we have determined that the *ref8* mutant is unable to convert *p*-coumarate to caffeate, suggesting that the mutant is defective in a gene required for the activity or expression of C3H. We have isolated the *REF8* gene using positional cloning methods and although many previous reports in the literature have suggested that C3H is a phenolase, we have found that the enzyme is actually a cytochrome P450-dependent mooxygenase. We have expressed C3H in a yeast host that concurrently overexpresses the gene for one of the two Arabidopsis P450 reductases. When yeast cells overexpressing the *REF8* gene are grown in media supplemented with *p*-coumarate, the *in vivo* bioconversion to caffeate can be readily detected by HPLC analysis of the yeast medium. Carbon monoxide difference spectra of microsomes from yeast expressing the *C3H* gene show a typical P450 spectral signature, which is absent in microsomes from yeast transformed with the control vector. *In vitro* enzymatic analysis of both wild-type and mutant C3H proteins is being conducted to characterize the fundamental properties of C3H. Preliminary kinetic data indicates that the K_m^{app} for *p*-coumarate may be higher than expected, suggesting that it may not be the optimal substrate for C3H, and that additional work may be needed to elucidate the role of this key enzyme in the phenylpropanoid pathway.

461 Putative THP gene identifies a novel gene family in *Arabidopsis thaliana*

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Mutagenized populations of *Arabidopsis thaliana* were screened for petal morphology mutants and a T-DNA insertional mutation was identified that produces abnormal flower organs. The petals in the *thin petal* mutants are narrow and curl inwards as the plant matures, in contrast to the wild type. The petal margins are uneven and jagged; sepals appear to be narrower as well.The combination of the phenotypes of these two whorls exposes the gynoecium precociously to outside pollen before *thp* pollen is released. As the plant matures, its growth habit becomes progressively more reclining.

We have rescued the T-DNA left border and its flanking plant sequence and now have a full length cDNA corresponding to the interrupted region. The putative *THP* gene maps to chromosome I and encodes a secreted protein of 541aa. Northern analysis shows transcripts of 2.05kb expressed in seedlings and flowers. The gene is a member of a small gene family of approximately 10 genes in *Arabidopsis*, coding for proteins with unknown function.

462 The placement of the embryonic shoot-root axis is disrupted in the *tilted axis* mutation

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The placement of the axes of symmetry (antero-posterior, dorso-ventral) is a defining event in the early embryogenesis of multicellular organisms. It organizes the body plan and determines where tissues will be specified. In plants little is known about this process. We have isolated a mutation in *Arabidopsis thaliana*, *tilted axis* (*til*), which causes an abnormal placement of the shoot-root axis with respect to the embryo-suspensor axis. The embryonic phenotype is quite variable, ranging from very abnormal patterns of cell divisions to almost normal-looking embryos. The mutation also shows a maternal effect: homozygous mutant embryos in a heterozygous carpel environment are able to recover and complete embryogenesis, while in a homozygous carpel environment a large fraction of them arrest development. A preliminary analysis of the mutant defects will be presented.

463 *LIGAND-LIKE PROTEIN (LLP)* is a large, previously unknown family of genes in Arabidopsis

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LIGAND-LIKE PROTEIN1 (*LLP1*) is a gene identified in microspore-derived embryos of Brassica napus. It encodes a small protein with 74 amino acids, including a predicted 23-AA signal peptide at its N-terminal. Sequence similarity between the first identified plant ligand peptide CLV3 and LLP1 led to the discovery of the highly conserved C-terminal LLP motif. The expression of *LLP1* in transgenic Arabidopsis is restricted to a small set of cells undergoing differentiation - at the edges of developing cotyledons, the periphery of the axillary buds and the maturation zone of the roots. Constitutive expression of LLP1 in Arabidopsis resulted in short roots and inflorescences but an increased number of branches. *LLP1* over-expression in roots does not affect root induction, but causes a dramatic consumption of the meristem. Based on these results, we propose that LLP1 is a putative ligand protein that triggers the differentiation of cells in the peripheral region of the meristem. The *LLP1* orthologs (*AtLLP1*) in Arabidopsis shares 68% amino acid sequence similarity with the LLP1 from Brassica. Based on the sequence features of *LLP1, AtLLP1* and *CLV3*, we identified 17 additional ORFs in the fully sequenced Arabidopsis genome (*LLP2 - LLP18*). All of them encode small proteins carrying an N-terminal signal peptide and a C-terminal conserved LLP motif. Because of their small size, all of them have been ignored by the current gene annotation analysis during genomic sequencing. Quantitative RT-PCR, Promoter::GUS fusion were used to establish the Expression profile of these genes, CaMV 35S promoter over-expression and T-DNA knockout analyses were carried out to study the function of these genes. The results obtained so far showed that most of these ORFs encode functional genes that are involved in regulating meristem activity or plant architecture. More detailed information about the functions of these genes will be presented during the conference.

464 The *BOBBER* gene encodes a protein with homology to an *Aspergillus* nuclear movement protein

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The *bobber* mutant of *Arabidopsis* was isolated during a screen for mutants that arrest during embryogenesis and fail to establish a correct pattern of *SHOOTMERISTEMLESS* (*STM*) expression. In wild-type embryos, *STM* is expressed exclusively in the developing shoot apical meristem, beginning at the transition from the globular to the heart stages of embryogenesis. In *bobber* mutant embryos, *STM* is expressed both in the cells of the developing shoot apical meristem and in cells of the presumptive cotyledons, which in wild-type embryos are free of *STM* expression. *bobber* mutants arrest at the globular stage of embryogenesis. Several other genes with embryonic expression are not misexpressed in *bobber*, indicating *BOBBER* is not required for general gene repression. The *BOBBER* gene has been positionally cloned and has been found to encode a homolog of a gene required for nuclear movement in *Aspergillus nidulans*. Morphological and expression data from both wild type and *bobber* mutant embryos will be presented.

465 Cytochrome P450s that control polar elongation of leaf

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The leaf is the key organ for a full understanding not only of plant morphogenesis but of its biodiversity. We showed previously that two genes of Arabidopsis are responsible for the polarity-specific expansion of leaves (Tsukaya et al., 1994; Tsuge et al., 1996). We succeeded in the molecular cloning of the *ROT3* gene, which regulates polar elongation in the leaf-length direction, showed that the *ROT3* gene encodes a cytochrome P450, CYP90C1 (Kim et al., 1998). We also succeeded to bio-design the leaf morphology of Arabidopsis from genetic manipulation of *ROT3* (Kim et al., 1999). Previous study suggested that the *ROT3* gene might be involved in a novel-steroid biosynthesis. To elucidate the hypothesis describe above, we firstly performed the expression study of *ROT3* gene in several mutants that were defected in light signal, gibberellic acid biosynthesis, brassinolide biosynthesis. Secondly, we analyzed the expression of *ROT3* mRNA by RT-PCR using *ROT3*pro::GUS transgenic plants that were treated or untreated with brassinolide intermediates (BLs). Finally, we analyzed the endogenous level and exogenous application of brassinolide intermediates in wt- and *rot3* mutant plants. The results of present study described above, suggest that CYP90C1 might be involved in a novel-branched pathway of brassinosteroid biosynthesis. In addition, we performed cloning of the *ROT3* homologue. This homologue shows approximately 51% homology to ROT3 with amino acid level. Phylogenetic tree indicates that the ROT3 homologue might be the same subfamily of ROT3, and designated as CYP90D1. Transgenic plants of up-regulated or down-regulated *ROT3* homologue were constructed. In order to investigate the function of this gene on leaf development, its molecular analysis is ongoing. We shall discuss about the molecular function of *ROT3* and *ROT3* homologue in leaf expansion process.

466 Promoter analysis of *CPC* for cell specific transcription in root epidermis

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Root epidermal cells differentiate to two cell types; either hair cells or hairless cells in *Arabidopsis*. The *caprice* (*cpc*) mutant has fewer root hairs than the wild type, and *CPC* encodes a Myb protein. The *35S::CPC* transgenic plants generate ectopic root hairs. These facts indicate that *CPC* is a positive regulator of the hair-cells' differentiation. Unexpectedly, however, *CPC* was preferentially transcribed in the hairless cells. In the *cpc* mutant, *CPC* was expressed in all the epidermal cells while in *35S::CPC* the expression level of *CPC* was decreased. The *werewolf* (*wer*) mutant has ectopic root hairs and *WER* encodes a Myb protein. In the *wer* mutant, the transcription of *CPC* was reduced in epidermis. These results indicate that the *CPC* expression is regulated negatively by CPC and positively by WER. To determine the regulatory elements for epidermis-specific transcription of *CPC* , we analyzed the transgenic plants in which a series of the truncated *CPC* promoters was combined to the *GUS* gene. By the examination of the GUS staining pattern of the roots of these transgenic plants, 1095 bp, 525 bp and 336 bp promoters show the activity in epidermis, although 268 bp and 238 bp promoters did not show the activity in epidermis. These results indicate that about 70 bp region between -268 to -336 is required for the epidermis-specific transcription of *CPC*. This region includes two putative Myb protein binding sites with a interval of about 20 bp. This finding raises the possibility that the CPC and WER proteins may directly regulate the transcription of the *CPC* gene.

467 Role of BEL1 like Homeobox genes in Arabidopsis

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BEL1 gene of Arabidopsis causes defective ovule development and is thus, believed to be an important regulator of ovule morphogenesis. Interestingly, BEL1 is also expressed in several other tissues which are apparently unaffected by a loss of BEL1 function. BEL1 belong to a family of twelve BEL1 Like Homeobox genes (BLH) in Arabidopsis. The predicted protein product of BLH genes contain domains that are involved in DNA binding, protein-protein interaction, nuclear localization which are shared between all members of the family. Expression analysis indicate that like BEL1 all members of BLH family tested are also expressed in several plant tissues. In addition, yeast two hybrid assay and in vitro analyses demonstrate that BLH proteins including BEL1 can selectively heterodimerize with specific KNAT proteins, a few members of which are known to be key regulators in meristem development. Thus, there is considerable potential for other BLH genes to be functioning with BEL1. Phylogenetic reconstruction implicates BLH2 as the gene most likely to share functional redundancy with BEL1. Consistent with the hypothesis, BLH2 has overlapping expression profile and interacts with the same KNAT partners in yeast two hybrid as that of BEL1. However, BLH2 also has its own tissue specific domains of expression that over lap or juxtapose with its putative interacting partners and hence, could have its own role to play in morphogenesis. Our recent genetic analysis in 35S-BLH2 lines show BLH2-KNAT interacting partners could indeed have a greater role in plant development.*BEL1BLHBLH2*

468 Investigation of Flower Development by Enhancer-Trap Mutagenesis

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Wild-type *Arabidopsis thaliana* flowers have four sepals, four petals, six stamens and two carpels. Two lines, TTV1 and TTV2, whose flowers show altered floral morphology have been generated by enhancer-trap mutagenesis (Sundaresan, V. *et. al*., 1995). The mutation causing TTV1 is recessive. The floral phenotype is highly variable; carpel and occasionally stamen number is increased, carpel-stamen chimeras are observed in the fourth whorl, carpels show a varying degree of fusion and tissue develops along their unfused edges as they age. This line shows GUS expression in the stigma, funicles, replum and vasculature of the valves in the silique. TTV1 contains a transposon insertion, identified by TAIL PCR, into an uncharacterised gene on chromosome 5. The predicted protein contains a ubiquitin-like N-terminal domain. TTV2 contains a trap insertion in the first exon of *CLAVATA1* (Leyser, H.M and Furner, I.J, 1992; Clark, S.E. *et. al*., 1993). on chromosome 1, and the TTV2 floral phenotype (caused by a recessive mutation) resembles that of a weak/intermediate strength *clavata1* allele. No GUS expression was observed in this line. Both mutations co-segregate with the observed phenotype.

References Clark, S.E. *et. al*. 1993, Development 119: 397-418 Leyser, H.M. and Furner, I.J. 1992, Development 116: 397-403 Sundaresan, V. et. al. 1995, Genes and Development 9: 1797-1810

469 Developmentally distinct MYB genes encode functionally equivalent proteins in *Arabidopsis*

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The duplication and divergence of developmental control genes is thought to have driven morphological diversification during the evolution of multicellular organisms.To examine the molecular basis of this process, we analyzed the functional relationship between two paralogous R2R3-MYB transcription factor genes, *WEREWOLF* (*WER*) and *GLABROUS1* (*GL1*), in *Arabidopsis*. The *WER* and *GL1* genes, which are known to positively regulate *GLABRA2* homeobox gene, specify distinct cell types and exhibit non-overlapping expression patterns during *Arabidopsis* development. WER controls root and hypocotyl epidermal cell development but does not control shoot trichome development. Its expression is limited to a specific subset of developing epidermal cells in the root and hypocotyl. On the other hand, GL1 does not control root and hypocotyl epidermal cell development but controls trichome formation. Also, *GL1* is expressed in the epidermal cells in developing shoot tissue. Nevertheless, a series of gene fusions, GL1 expressed under the control of the *WER* regulatory region or WER expressed under the control of the *GL1* regulatory region, were able to complement the *wer* or *gl1* mutant phenotype, respectively. These results show that *WER* and *GL1* encode functionally equivalent proteins, and their unique roles in plant development are entirely due to differences in *cis*-regulatory sequences. Similar experiments with a distantly related MYB gene (*MYB2*) showed that its product can not functionally substitute for WER or GL1 when it is expressed under the control of *WER* regulatory region or under the control of *GL1* regulatory region. Furthermore, analysis of the WER and GL1 proteins shows that sequences correspond to specific functional domains. These results provide new insights into the evolution of the MYB gene family in Arabidopsis, and, more generally, they demonstrate that novel developmental gene function may arise solely by the modification of *cis*-regulatory sequences.

470 Role of *WUSCHEL* in specifying stem cell identity in the *Arabidopsis* shoot apical meristem.

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Higher plants form virtually all of their aerial organs postembryonically and therefore require a continuous supply of cells. This is provided for by stem cells (SC) located at the tip of the shoot apical meristem (SAM). Stem cell identity is specified by the putative homeodomain transcription factor WUSCHEL (WUS). *WUS* mRNA is expressed in a central cell group underneath the SC, termed the organizing centre (OC). Thus, *WUS* acts non-cell-autonomously.

By which mechanism does *WUS* expressed in the OC specify the overlying neighbours as SC? Is WUS protein transported there or does it act via intermediate genes that it activates in the OC? To address this question, we localized WUS protein by expressing a translational fusion of WUS to β-Glucuronidase (GUS) under the endogenous *WUS* promotor and staining for GUS activity. The fusion protein was functional as shown by complementation of the *wus* mutant phenotype. Our results indicate that WUS protein is not transported into the SC, but remains in the cells of the OC. This suggests that its non-cell-autonomous action is mediated by products of downstream target genes activated in OC cells.

To identify possible target genes, we isolated several transcripts that were upregulated after induction of *WUS* activity. The expression patterns of the corresponding genes were analyzed by *in situ* hybridization and are consistent with their being *WUS* targets: Expression was found in or adjacent to the cells of the OC, and the expression domains were increased in plants with an enlarged domain of *WUS* expression. None of the isolated genes shows homology to proteins of known function. Their role in specifying SC identity is currently being analyzed by ectopic expression and a screen for insertion mutants.

471 Characterization of the gibberellin signal transduction pathway that represses expression of embryonic identity in the *pkl* mutant of Arabidopsis

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PKL encodes a CHD3-chromatin remodeling factor that is necessary for repression of embryonic identity in Arabidopsis. Primary roots of *pkl* seedlings that express embryonic differentiation characteristics are green and tuberous and are referred to as “pickle roots”. Penetrance of the pickle root phenotype is dependent on gibberellin (GA). When *pkl* seedlings are germinated on synthetic media, the penetrance of the pickle root phenotype ranges from 1% to 5%. Germination in the presence of a chemical inhibitor of GA biosynthesis such as uniconazole dramatically increases penetrance of the pickle root phenotype to greater than 80%. Based on these observations, we propose that GA acts to inhibit expression of embryonic identity in *pkl* seedlings via an as yet uncharacterized GA signal transduction pathway that is PKL-independent. Although abscisic acid (ABA) acts antagonistically to GA with respect to germination, we have shown that expression of the pickle root phenotype is largely ABA-independent. Germination in the presence of ABA does not substantially increase penetrance of the pickle root phenotype. In order to identify components of this GA signal transduction pathway, we are screening for mutants that enhance the penetrance of the pickle root phenotype in the presence of GA. We have generated ~3000 M3 lines from EMS-mutagenized *pkl* seeds. At present, we have screened approximately 1600 of these M3 lines and identified ~20 possible enhancers. One enhancer exhibits 100% penetrance of the pickle root phenotype in the presence of 10^{-6}M GA_3 whereas several other enhancers exhibit 50-80% penetrance of the pickle root phenotype in the presence of 10^{-6}M GA_3. Characterization of these enhancers is in progress.

472 Embryonic Control of Epidermal Cell Patterning in the Root and Hypocotyl of Arabidopsis

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A fundamental feature of development in multicellular organisms is the specification and patterning of distinct cell types. We study the position-dependent formation of cell types in the root and hypocotyl epidermis of Arabidopsis as a simple model for understanding cell patterning in plants. During epidermis development in these organs, cells that lie in the intercellular space between underlying cortical cells preferentially differentiate into specialized cell types (root hair cells in the root and stomatal complexes in the hypocotyl), whereas cells lying over a single cortical cell develop as unspecialized epidermal cells (non-hair cells in the root and non-stomatal cells in the hypocotyl). To understand the developmental origin of this process, we have examined the embryonic control of epidermal cell patterning in the Arabidopsis seedling. We have employed the *GLABRA2* (*GL2*) gene, a known cell-type-specific transcription factor, to accurately assess the origin and the regulation of the epidermal cell specification mechanism. The *GL2* expression pattern has been determined throughout embryogenesis using *in situ* RNA hybridization and by employing a sensitive *GL2::GFP* reporter construct. Furthermore, we examined the embryonic effect of mutations in known regulators of the seedling epidermal patterning, including the *TRANSPARENT TESTA GLABRA* (*TTG*), *WEREWOLF* (*WER*), and *CAPRICE* (*CPC*) genes. We also analyzed the origin and patterning of the epidermal cells at the junction between the root and hypocotyl. Our results suggest that the positional information that establishes the epidermal pattern originates at an early stage of embryogenesis. Additional information about our lab’s research is available at our website - http://biology.lsa.umich.edu/research/labs/schiefel/

473 Delayed Floral Organ Abscission Mutant *dab4* Displays Pleiotropic Phenotype

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To gain an understanding in the process of abscission in Arabidopsis, we screened the Wisconsin T-DNA insertion lines for delayed abscission of floral organs. Plants that maintained their petals beyond position ten on the inflorescence were selected. *dab4 (delayed abscission 4)* was selected for a delay in abscission beyond position 20. Wild type plants usually discard floral organs by position 7. *dab 4* also displays phenotypes other than delayed petal abscission, showing reduced number of primary inflorescence stems, severely delayed inflorescence meristem arrest, and male sterility due to the lack of pollen sac dehiscence. As a result, *dab4* inflorescences grow up to four feet high and produce more than 200 flowers. In addition, *dab4* mutants have dark green leathery leaves. To determine the molecular nature of *dab4* , we examined the number of insertions with southern blot and isolated the T-DNA junctions using tail PCR with degenerate primers AD-1 and AD-2 in combination with T-DNA borders. Both approaches indicate that two unlinked T-DNA insertions are present in *dab4* . We will present the anatomical, physiological, and preliminary molecular characterization of *dab4* .

This work was funded by USDA grants 9835301-6764, 0035301-9085, and WIS04409 Corresponding author: spatters@facstaff.wisc.edu

474 The *hydra* mutants of Arabidopsis demonstrate an essential role for bulk membrane sterols in determining hormone signalling integrity and cell fate control

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The *hydra1* and *hydra2* mutants of Arabidopsis are embryonic-defective, and have almost identical pleiotropic seedling phenotypes. They are characterized by multiple patterning and cell identity defects, including supernumerary cotyledons of mixed phase, disorganized and multiple shoot meristems, widened hypocotyls with loss of radial patterning and disrupted vascular development, ectopic root hairs, and reduced primary root growth and lateral root formation. *hydra2* is defective in the maintenance of columella cell fate. We have cloned the *HYDRA1* gene, which encodes a delta8-delta7 sterol isomerase enzyme. We have also found that *HYDRA2* is allelic to *FACKEL,* a gene coding for sterol C-14 reductase, the enzyme immediately preceding the delta8-delta7 sterol isomerase in the sterol biosynthetic pathway. Analysis of sterol content in the mutants confirms a role for these genes in bulk sterol production. We present evidence that *hydra* mutants exhibit defects in both auxin and ethylene signalling, and we propose that sterols play an essential role in determining signalling integrity, perhaps by maintaining membrane fluidity, permeability or sterol/lipid-protein interactions in signalling, including brassinosteroid detection (these mutants contain very low levels of brassinosteroids but cannot be rescued by exogenous brassinosteroid application). This represents the first direct evidence for a role for plant sterols in maintaining the integrity of auxin and ethylene signalling pathways required for correct cell patterning and identity.

Refs.Topping JF, May VJ, Muskett PR and Lindsey K (1997) Mutations in the *HYDRA1* gene of Arabidopsis perturb cell shape and disrupt embryonic and seedling morphogenesis. *Development* 124: 4415-4424. Schrick K, Mayer U, Horrichs A, et al. (2000) *FACKEL* is a sterol C-14 reductase required for organized cell division and expansion in Arabidopsis embryogenesis. *Genes Dev.* 14: 1471-1484.Jang JC, Fujioka S, Tasaka M, et al. (2000) A critical role of sterols in embryonic patterning and meristem programming revealed by the *fackel* mutants of *Arabidopsis thaliana*. *Genes Dev.* 14: 1485-1497.

475 The MONOPOLE gene encodes a GATA-factor involved in the regulation of cell fates at the basal pole of the early embryo

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In Arabidopsis, formation of the root meristem is initiated at the basal pole of the 16-cell embryo. The embryonic root is formed through an almost invariant sequence of cell divisions involving the uppermost suspensor cell as well as the adjacent cells of the embryo proper. From a large-scale mutant screen for embryos with altered morphology we have recovered four recessive allelic mutations, designated MONOPOLE, which interfere with this process. The basal cells of MONOPOLE mutant embryos divide aberrantly producing fewer and irregularly arranged daughter cells. As a consequence, no root primordium is recognizable by anatomical criteria. However, MONOPOLE mutants are capable of forming a functional root later in embryogenesis. A molecular marker for the quiescent center of the root is expressed in the central cells of the embryo suggesting that the embryonic root is initiated ectopically. We have cloned the MONOPOLE gene in a map-based approach and found that it encodes a protein with high similarities to transcriptional regulators of the GATA family. We are presently determining the expression pattern of MONOPOLE RNA in the early embryo.

476 The role of *PINHEAD* in embryonic patterning and phyllotaxy

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In *pinhead* mutants, the indeterminate shoot axis of the embryo, which includes the shoot apical meristem, is transformed to a determinate state. This phenotype is incompletely penetrant, perhaps due to the activity of the related and partially redundant gene *ARGONAUTE*. Double mutations in *PINHEAD* and *ARGONAUTE* cause synergistic effects on development, leading to embryonic arrest prior to attainment of bilateral symmetry. We present evidence that the *SHOOT MERISTEMLESS* transcript is not translated in *argonaute pinhead* double mutants. These data suggest that *PINHEAD* and *ARGONAUTE* excersize post-transcriptional control over *SHOOT MERISTEMLESS*. In addition, we show that normal patterns of gene expression are disrupted in the *argonaute pinhead* double mutant, indicating that embryonic patterning is perturbed. This phenotype clarifies a role for *PINHEAD* in providing positional information in the radial dimension to the developing plant. Consistent with this hypothesis, we show that those *pinhead* single mutants that initiate "normal" meristems suffer from disorganized phyllotaxy (the radial positioning of leaves). *PINHEAD* expression is the earliest reported marker of leaf initiation. Here we demonstrate that *PINHEAD* transcript accumulation predicts the positions of at least four incipient leaf primordia that are morphologically indistinguishable. These data together indicate that *PINHEAD* contributes to the radial positioning of leaves.

K. Lynn et al., *Development* 126, 469-81 (1999).J. R. McConnell, M. K. Barton, *Developmental Genetics* 16, 358-366 (1995).B. Moussian, H. Schoof, A. Haecker, G. Jurgens, T. Laux, *Embo* J 17, 1799-809 (1998).

477 The role of *ASYMMETRIC LEAVES1* and *2* in Arabidopsis leaf development

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The leaves develop from the shoot apical meristem and generally exhibit bilaterally symmetrical and flattened architecture. A leaf of wild type Arabidopsis shows bilateral symmetry in two respects; the position of the serrated margins and venation patterns of leaf blades are symmetry. As the midvein exists at the center of leaf blade along the longitudinal axis, it may play an important role in the formation of symmetrical leaves. The *asymmetric leaves1* (*as1*) and *asymmetric leaves2* (*as2*) mutants in *Arabidopsis thaliana* produced asymmetrical leaf lobes. The *as1* and *as2* leaves failed to produce a thick and distinct midvein. The secondary veins were formed at the left-right asymmetrical pattern in the leaf blade and did not join but exist parallel to the primary vein in a petiole. The *as* mutations caused the accumulation of transcripts of meristem-related homeobox genes *KNAT1*, *KNAT2* and *KNAT6* in leaves. We also observed the significantly higher efficiency of shooting on rosette-leaf sections of *as* mutants *in vitro*. We isolated the *AS2* gene that showed to encode a protein that belonged to a new family with a leucine-zipper motif. *AS1* has been shown to encode a domain which is similar to the myb repeat. We showed that genetic and molecular interaction between *AS1* and *AS2*. These observations indicate that *AS1* and *AS2* repress meristem-related homeobox genes in the leaf cells, and are involved in establishment of a prominent midvein and venation and regulate formation of symmetrical leaf lamina.

478 CRE1/WOL cytokinin receptor regulates vascular morphogenesis of the Arabidopsis root

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The developmental ontogeny of the vascular system (consisting of xylem, phloem and [pro]cambium) is poorly understood despite its central role in plant physiology. We are studying the genetic control of vascular patterning during root development in Arabidopsis. We have recently determined the cell lineage relationships of the vascular tissue in the root meristem: xylem cell lineages are specified close to the underlying quiescent center, whereas phloem and procambium are established through a set of asymmetric cell divisions further up. Consequently, we have been searching and characterizing for mutations that affect this pattern. The primary effect of the *wooden leg* (*wol*) mutation is the lack of the formative cell divisions required for the organization of the vascular tissue (Scheres et al. Development 121: 53). We have determined that the *WOL* gene codes for a putative signal transducer with a histidine kinase activity (Mähönen et al. Genes Dev 14: 2938). It is expressed specifically in the vascular tissue from the early stages of embryogenesis on. Recently, Inoue et al. (Nature 409:1060) showed that CRE1/WOL is a true cytokinin receptor. Taken together, this indicates that cytokinins regulate the procambial cell divisions of the Arabidopsis root through a specific signal transduction pathway. Recent progress in the further characterization of this pathway using molecular and genetic approaches will be presented.

479 *PRESSED FLOWER* promotes the proliferation in L1 cells of the lateral region of a flower and floral organs

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Structure of a flower predicts the presence of two crossed axes, the abaxial-adaxial axis and the lateral axis in relation to the inflorescence meristem. Floral organs could have the same two axes in relation to the floral meristem. In the Arabidopsis mutant, *pressed flower* (*prs*), growth of the lateral sepals is repressed. Both size and shape of abaxial and adaxial sepals are normal, but the marginal cell files are missing. *PRS* encodes a putative transcriptional factor with a WUSCHEL-like homeodomain. *In situ* hybridization showed *PRS* to be expressed in L1 cells of the lateral regions of both flower primordia and floral organs, indicating this expression pattern cleared the presence of lateral axis. These patterns are mostly consistent with the genetic defects of prs, suggesting that *PRS* is involved in the cell proliferation at the lateral regions of flower primordia and floral organs.

To investigate the function of PRS as a regulator of cell proliferation, we analyzed the effects of a gain-of-function mutation of *PRS*. In 35S:*PRS* transgenic plants, multicellular bulges with trichomes were observed on the stem. Multicellular bulges without trichomes were also formed on the pedicel. We interpret these bulges are the result of ectopic and over proliferation of the epidermal cells. On the sepals, white wrinkle structures were observed. Transverse sections showed the structures to be outgrowths of epidermal cells. It is worth noting that the margin of wild-type sepals is made of similar outgrowth of epidermal cells. These aberrant proliferation of epidermal cells in various organs observed in the transgenic plants suggests that PRS functions to promote proliferation of L1 cells as a transcriptional factor.

Our previous studies and this report show that (1) *PRS*, promoting the cell proliferation, is expressed in L1 cells at lateral region of a flower primordium, which will be recruited to the lateral sepals; the mutation in *PRS* cause the defects in the development of lateral sepals, and that (2) *PRS* is also expressed in the lateral margin of sepals and promote the cell proliferation; in the mutant, the marginal cells of sepals are absent.

480 Developmental functions of auxin: MONOPTEROS dependent regulation of developmental genes

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The Arabidopsis MONOPTEROS (MP) gene has a central role in embryo axis and vascular strand formation (1). MP encodes a transcription factor of the 'Auxin Response Factor' (ARF) family, which can regulate the expression of auxin-induced genes by binding to conserved promoter elements (2, 3). Several lines of evidence have implicated auxin in cell pattern formation (4). MP could therefore relay auxin signals in embryo axis formation and vascular differentiation, but its target genes are unknown.We have adopted three strategies to identify genes downstream of MP. First, we have assessed the expression profiles of a large number of genes in vascular differentiation and auxin transport. Among the genes whose expression is correlated to MP gene activity, we found members of the HD-ZIP transcription factor family and members of the PIN family of presumptive auxin efflux carriers. We will report details of the MP controlled expression of AtHB8, an early vascular transcription factor (5). Second, we have identified a larger collection of potentially MP dependent expression profiles on DNA microarrays. Downstream genes include members of the AUX/IAA, HD-ZIP and GH3 families. Third, in order to identify immediate targets of the MP, we will determine transcript profiles in the background of posttranslationally controlled MP gene activity.[1] Przemeck et al. (1996). Planta 200: 229-237. [2] Hardtke and Berleth (1998). EMBO J 17: 1405-1411.[3] Ulmasov et al. (1997). Science 276: 1865-1868.[4] Berleth and Mattsson (2000) Curr. Op. Pl. Biol. 3: 406-411.[5] Baima et al. (1995). Development 121: 4171-4182

481 A dwarf Arabidopsis mutant isolated from activation-tagging lines shows exaggerated epinastic leaves

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We isolated a semidominant dwarf mutant, #4094, from 10,000 activation-tagging lines of Arabidopsis. Heterozygous #4094 plants were dwarf, produced only a small amount of seeds, and had very curly leaves as a result of strong epinasty. These abnormalities appeared more severe in homozygous #4094 plants. Hypocotyls of the mutants in the dark grew spirally or frizzily, although length of them was essentially the same as wild type. In contrast, when grown under white light, they were significantly longer than those of wild type. Spiral or frizzy growth was also seen in various organs of the mutant such as stems and fruits. In these organs cortex and epidermal cells showed abnormal shapes, and enlarged more than those of wild type. It is known that ethylene induces long hypocotyls and epinastic leaves under white light. But treatment with an ethylene precursor, ACC, of wild type plants under white light could not mimic the mutant phenotypes, and AVG, an inhibitor of ethylene biosynthesis, had no effects on leaf epinasty and length of hypocotyls of the mutant. In addition, etiolated seedlings of the mutant did not show triple responses. These suggest that the mutant phenotypes including long hypocotyl under white light and leaf epinasty, are not caused by ethylene over-production. Near the enhancer sequence in the T-DNA end, we found a gene encoding a Ser/Thr protein kinase which belongs to NAK subfamily (Hardie, 1999). Northern analysis revealed that mRNA of this gene accumulated more than 100-fold in #4094 plants compared to wild type. RT-PCR showed that this gene expressed in various organs in wild type, such as root, stem, leaf, flower, and fruit, at very low level. These results suggest that the abnormalities of #4094 plants result from activation tagging of this kinase gene. Protein kinases of NAK subfamily have conserved kinase domains flanked by short non-kinase domains on both sides. In Arabidopsis, there are more than 40 members of this subfamily, but almost nothing is known about their functions.

482 Identifying downstream targets of the floral homeotic *APETALA3* and *PISTILLATA* genes

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Although the floral homeotic genes described in the ABC Model are well accepted as the master regulators in the specification of different floral organ identities, how they actually lead to the development of the different organs (i.e. their downstream pathways) remains unknown. Despite considerable effort, only a few genes regulated by the floral homeotic genes have been identified to date. We are using multiple approaches in order to identify downstream targets of the B-class genes, *APETALA3* and *PISTILLATA*, which are required for petal and stamen organogenesis. Candidate genes are being isolated by activation tagging, gene trapping, microarray analysis and chromatin immunoprecipitation. Eight thousand activation tagged lines have been screened for abnormal floral morphology. Several putatively tagged abnormal floral mutants have been recovered, and the genes responsible for these phenotypes are being isolated. Gene trap lines containing the *GUS* reporter gene with a splice acceptor site[1] are being screened for specific patterns of expression in flowers. A summary of the patterns of expression conferred by the *GUS* insertions and the sequences of the corresponding genes will be presented. We are also using microarray analysis to conduct a broader survey of genes whose expression is affected by AP3 and PI activity. DNA microarrays representing approximately 9,000 Arabidopsis ESTs are being screened with probes corresponding to mRNAs from different *AP3*/*PI* loss- or gain-of-function mutants. Preliminary results suggest that a relatively small population of genes are regulated by AP3 and PI. In addition, we are conducting a more specific screen for direct targets of AP3/PI by carrying out immunoprecipitation of chromatin fragments that are bound to AP3/PI *in vivo* using AP3/PI-specific antibodies.

[1] Sundaresan V. et al. (1995) Genes & Development 9:1797-1810.

483 Arabidopsis Root Meristem Organization Depends on a Transcription Factor Expressed in the Quiescent Center

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In *Arabidopsis*, the root apical meristem is comprised of stem cells for each root tissue type, which surround a core of four mitotically quiescent cells (the quiescent center, or QC). To determine the role of the QC in maintaining meristem pattern, we have isolated an enhancer trap that is expressed exclusively in these cells. Sequence analysis reveals that the locus encodes a member of a well-known transcription factor family. Although two independent hypomorphic alleles do not display defects in root growth, the QC and surrounding stem cells appear disorganized in a fraction of homozygous individuals. The highly specific expression pattern has been confirmed by promoter-GFP fusion, and cannot be detected in the root tip until 3-4 days post germination. Embryos and young lateral roots do not show expression, indicating that the gene product must act in the maintenance, rather than initiation, of meristem organization.

484 *SHORT VALVE*, a gene encoding a ribosomal protein L24 homolog is involved in the gynoecium and flower bud development

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The gynoecium of *Arabidopsis* is composed of four distinct parts, stigma, style, ovary and gynophore along the apical-basal axis postulated in the gynoecium structure. We have isolated a novel gynoecium mutant, *short valve-1* (*stv-1*), in which the basal region of the ovary is replaced by the gynophore, resulting in the reduced size of the ovary. Similar phenotypes are reported in *pid-8*, *ett-2* and *fil-1*, suggesting that these genes involved in the differentiation of the ovary. To understand the genetic interaction of them, these mutants were crossed each other to generate the double mutants. Each double mutant displayed severer phenotypes than every parental line. Especially *stv-1 pid-8*, *stv-1 ett-2*, *stv-1 fil-1* and *pid-8 fil-1* double mutants had *pin1*-like inflorescence without any flower buds, indicating that these genes also worked together in the formation of a flower bud and that *STV* might be involved in the auxin transport or response.

We cloned the *STV* locus by T-DNA tagging and showed that *stv-1* was caused by a 10-kb deletion on chr. III, which contained five predicted genes. Complementation analysis with each predicted gene and screening for knock-out lines demonstrated that *STV* encoded a homolog of the ribosomal protein L24. There are two possibilities to explain the role of *STV*. One explanation is that decrease of protein synthesis activity affects the gynoecium development. The other explanation is that this mutation affects gynoecium-specific development. We will also show the result of expression analysis of *STV*.

485 Function of *scarecrow*-like genes in root radial patterning

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The *Arabidopsis* primary root has a highly stereotyped cellular structure, which is initially patterned during embryogenesis. This structure consists of concentric cylinders of specific cell types that ensheath the vascular bundle. From inside to outside, these single-cell layers are the pericycle, endodermis, cortex, epidermis, and, at the tip of the root, the lateral root cap. These cell layers are specifically lineally related to each other: During embryogenesis the pericycle and vascular tissue form from the procambium, the endodermis and cortex derive from the ground meristem, and the epidermis and lateral root cap arise from the protoderm. While cell lineage in the primary root predicts cell fate, cell-ablation studies have shown that positional information is critical for proper cell specification.

Two genes known to be important in the formation of root radial pattern during embryogenesis are *SHORT-ROOT* and *SCARECROW*. In the *short-root* mutant, the endodermal cell layer is missing, and in the *scarecrow* mutant, the endodermis and cortex are present in the form of a single, hybrid cell layer. Both the *SHORT-ROOT* and *SCARECROW* genes belong to the GRAS family of putative transcription factors, which is unique to plants. We reasoned, therefore, that additional members of this family may also play a role in radial pattern formation and/or cell fate specification in the root. We will present an analysis of T-DNA insertion mutants and plants exhibiting RNA interference for various GRAS family members. One of these genes may be important for transducing positional signals.

486 A MYB Transcription factor Promotes Mucilage Secretion in the Seed Coat Epidermis

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The Arabidopsis seed coat epidermis differentiates into highly specialised cells that form raised columellae and fill with mucilage. The development of these cells requires the coordinated regulation of cell shape, mucilage deposition, secondary cell wall synthesis and primary cell wall degradation. We have charcterised the role of a MYB transcription factor in seed coat development through the phenotypic analysis of three independent transposon insertion alleles. We have determined that Arabidopsis seed mucilage consists primarily of a linear rhamnogalacturonan polymer, and that the secretion of this is reduced in the MYB mutant seed coats. In addition to its role in mucilage deposition this gene is also required for the breakdown of the primary cell wall. Comparison with the known seed coat mutants *ttg-1* and *gl2-1* suggests that the *myb* mutant testas are less severely affected and show a novel phenotype. Our analysis of the *myb* and *ttg-1* mutants have led to new insights into the functions of these genes during development and germination.

487 The *Arabidopsis* subunit 3 of the COP9 signalosome is encoded by *FUS 11* and involved in multifaceted developmental processes

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The COP9 signalosome is a nuclear enriched multisubunit protein complex initially defined as a repressor of photomorphogenic development in *Arabidopsis* . It is highly conserved among diverged organisms and shares remarkable similarity with the lid subcomplex of the 26S proteasome in both subunit composition and one to one subunit sequence. We have shown that it regulates the degradation of specific substrates probably by replacing the lid subcomplex of the 26S proteasome. The *Arabidopsis* subunit 3 of the COP9 signalosome (CSN3) is encoded by the *FUS 11* gene. The *fus11* locus contains a mutation at the 3' splicing junction of the 9th intron thus results in an alternative splicing and early termination of the reading frame. Overexpression of the *CSN3* cDNA complements the *fus11* mutation. The CSN3 protein contains a PCI domain at C-terminals and a Leucine zip domain at the N-terminals and shares 42% and 31% identities with its human and *Drosophila* counterpart respectively. Partial reduction-of-function strains of CSN3 obtained by gene co-suppression causes accumulation of ubiquitinated proteins and developmental defects in pattern formation, organ boundary defining, phyllotaxy, and organ identity control, suggesting a critical role of the COP9 signalosome regulated protein degradation in plant development.

488 The *arrested development* Mutants Alter Meristem Function and Cell Proliferation in Leaf Margins

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The *arrested development* (*add*) *1* and *3* mutants cause temperature dependent loss of normal shoot development. Homozygotes for *add1* lose normal meristem organization and function at high temperature, while *add3* homozygotes fail to complete a normal program of leaf formation. The *add1* mutation was induced by T-DNA integration and the genomic region surrounding the site of T-DNA insertion has been sequenced. A well-modeled gene has been identified at this site and an expressed sequence tag recovered corresponding to the 3' end of this gene. The site of T-DNA insertion has been mapped to a resolution of 100 base pairs and indicates that the T-DNA integration site falls in the last exon of the gene model. The results of transformation rescue and expression experiments will be presented providing supporting evidence that the identified gene model encodes ADD1 function. Extensive histological analysis of up-shifted meristems and leaves will also be presented, confirming the role of ADD1 in maintenance of meristem structure and adding additional support to the “meristem imposes adaxial” model for the specification of leaf dorsiventrality.

A high-resolution recombination breakpoint map has been generated to provide the precise location of the *ADD3* locus on the extant physical and sequence map of the Arabidopsis genome. A bacterial artificial chromosome (BAC) contig has been mapped to this region and a three BAC clone sub-contig spans the region between two markers known to flank the *ADD3* gene. Based on the derived recombination rate for this region, a candidate gene has been identified for the *ADD3* gene. Results of transformation rescue experiments and the sequence of wild type and mutant alleles of the candidate gene will be presented that suggest that this candidate is the *ADD3* gene. Extensive histological analysis of *add3* leaves indicates that up-shifted plants lose normal spongy mesophyll proliferation in the marginal region of leaves. Identification of a 98% identical orthologue of the *ADD3* candidate suggests that the *add3* mutation is due to temperature sensitive genetic redundancy, an outcome predicted by our theoretical work supporting the Duplication, Degeneration and Complementation model of duplicate gene evolution.

489 A Fate Map of the Two Apical Cell Stage Arabidopsis Embryo

Alexandria Saulsberry[1], Paula R. Martin[1], Tim O'Brien[1], Leslie E. Siebruth[2], F. Bryan Pickett[1]
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Alexandria Saulsberry[1], Paula R. Martin [1], Tim O'Brien [2], Leslie E. Seibruth[3], and F. Bryan Pickett[1*][1]Dept. of Biology, Loyola University of Chicago, 6525 N. Sheridan Rd. Chicago, IL 60626, USA[2]Dept. of Mathematics and Computer Science, Loyola University of Chicago[3]Dept. of Biology, University of Utah, Salt Lake City, UT 84112, USA[*]Author for Correspondence (fpicket@luc.edu)Lineage analysis of the pattern of cell division in the early *Arabidopsis* embryo may provide insight into the likely contribution of mosaic and regulative developmental processes to plant embryogenesis. A transgenic system placing the Cre recombinase under heat shock regulation and flanking a constitutive marker gene (35S::GUS) with the *Plox* binding site for Cre has been used to construct GUS+ | GUS- genetic chimeras. Embryos with two apical cells were heat-shocked and the resulting plants were scored for GUS- sector extent in the mature cotyledons and leaves of the plant. The resulting fate map demonstrates that the daughters of the first two apical cells tend to contribute primarily to one cotyledon or another and their physically associated true leaves. This result indicates that patterns of early cell division limit the future developmental potential of these cells. However, GUS- clones are found shared between all regions of the mature plant, suggesting that there is no strict lineage restriction imposed on the daughters of the first apical cells. These results indicate that early apical cell divisions correlate with the establishment of embryonic axes, although whether these divisions partition mosaic regulatory information, are a response to extraembryonic cues, or result from a combination of both developmental processes remains an open question.

490 Isolation and characterization of the major plastidial acyl-CoenzymeA synthetase from Arabidopsis thaliana

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Acyl-CoenzymeA synthetases (ACS, EC 6.2.1.3.) catalyze the formation of fatty acyl-CoAs from free fatty acid, ATP, and CoenzymeA. The products of the reaction, fatty acyl-CoAs, are utilized in the synthesis of lipid molecules, including membrane glycerolipids and triacylglycerols. ACSs are therefore integral to plant lipid metabolism. ACS activity has been localized in membranes of various organelles, including oilbodies, peroxisomes, mitochondria, microsomes, chloroplasts, and plastids. Plastids are the site of all *de novo* fatty acid synthesis. Before newly synthesized fatty acids are exported from the plastid for subsequent incorporation into membrane lipids or triacylglycerol, they must first undergo activation to fatty acyl-CoA esters by an ACS. We were interested in characterizing the plastidial ACS from *Arabidopsis* because of the significant role it plays in de novo fatty acid synthesis. Our laboratory identified and cloned a family of eleven ACSs. Here, we describe the identification and characterization of one ACS isoform, *AtACS6B*. Isolation and analysis of a mutant containing a T-DNA interruption in *AtACS6B* has led to our conclusion that this isoform encodes the major chloroplastic ACS.

491 Non-cell autonomous function of SHORT-ROOT in root radial pattern formation: 1. Molecular basis for intercellular signaling

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Positional information plays a pivotal role in the formation and maintenance of plant developmental patterning. The root radial pattern is generated through stereotyped division of a set of initial cells in the meristem and subsequent acquisition of cell fate. This process has been shown to be primarily dependent on positional information. While the paradigm of position-dependent cell fate determination has been accepted for root pattern formation, little is known about the signaling mechanism.

Analysis of radial pattern formation in the *Arabidopsis* root indicated a non-cell autonomous function of the *SHORT-ROOT (SHR)* gene. *SHR* encodes a putative transcription factor and is transcribed in the stele but is necessary for the formation and specification of the endodermis layer.

Two different scenarios can in principle account for non-cell autonomous action of a transcription factor in plants: the activation of downstream genes coding for secreted ligands, or a direct movement of the transcription factor itself through plasmodesmata.

Two independent methods are here used to observe the endogenous localization of the SHR protein. Results will be shown that reveal novel insights into the mechanisms responsible for the root radial positional information.

492 *grv2*: A viable embryonic mutant characterized by the presence of enlarged and highly vacuolated cells at the apex

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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 that it is apparently agravitropic. We have complemented the mutant phenotype with a cosmid containing two candidate genes.

493 The *LOB* gene family in Arabidopsis

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The *LATERAL ORGAN BOUNDARIES* (*LOB*) gene was identified as an enhancer trap insertion that showed GUS reporter gene expression at the base of all lateral organs. *LOB* encodes a predicted 20kD peptide with no recognizable functional motifs. LOB contains a highly conserved amino-terminal domain that is present in 36 other Arabidopsis proteins and in proteins from a variety of other plant species. The *LOB* gene family appears to be plant specific, as related genes have not been detected outside of plant databases. The expression of *LOB* at the junction between the shoot apical meristem and lateral organ primordia suggests a potential role for *LOB* in establishing a boundary between the SAM and initiating lateral organs. Loss of function *LOB* mutants have no detectable phenotype, suggesting that *LOB* is functionally redundant. Ectopic expression of *LOB* leads to alterations in the size and shape of leaves and floral organs and causes male and female sterility. *LOB* is mis-expressed in transgenic plants that ectopically express either *STM* or *KNAT1*, suggesting that *LOB* acts downstream of *KNOX* gene signaling.

494 ASYMMETRIC LEAVES1, an Arabidopsis gene that is involved in the control of cell differentiation in leaves

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During leaf development, the formation of dorsal-ventral and proximal-distal axes is central to leaf morphogenesis. To investigate the genetic basis of dorsoventrality and proximodistality in the leaf, we screened for mutants with defects in leaf morphogenesis. Here we describe phenotypes of three newly isolated *asymmetric leaves1* (*as1*) mutants, *as1-101, as1-102* and *as1-103*, which are in the Landsberg *erecta* (L*er*) genetic background. In addition to the leaf phenotypes described previously, these alleles display more phenotypes that were not observed previously. These include: 1) a portion of rosette leaves with petiole growth underneath leaf lamina, displaying a lotus-leaf-like structure; 2) leaf vein branching in the petiole; and 3) leaf lamina with epidermis similar to that on the petiole. *AS1* gene has been identified and the functional analysis revealed that this gene negatively regulates *KNOX* gene family during the establishment of proximodistal axis in leaf development (Byrne et al. 2000). Our phenotypic analysis at the cellular level indicates that *AS1* may control the timing of cell differentiation in leaves. Loss of function in *AS1* causes the cell differentiation being prolonged as shown in lateral vein and minor vein development in cotyledons and rosette leaves. Furthermore, we have analyzed *AS1* over-expression in the wild-type L*er* plants. The 35S::AS1 transgenic plants produced long and narrow rosette leaves. In addition, rosette leaves and cauline leaves in the transgenic plants contain increased minor veins and dramatically increased trichomes. These results provide further evidence that *AS1* has functions in the control of cell differentiation in leaves.

495 The *CUP-SHAPED COTYLEDON1* gene of Arabidopsis regulates shoot apical meristem formation

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Shoot apical meristem (SAM) formation ad its function are essential for the development of higher plants. *CUC1* and *CUC2* are functionally redundant genes that are important for embryonic SAM formation and organ separation in Arabidopsis. *CUC* genes are thought to promote embryonic SAM formation through transcriptional activation of the *SHOOT MERISTEMLESS* (*STM*) gene, because *STM* mRNA was not detected in the *cuc1 cuc2* double mutant. In this study, we cloned the *CUC1* gene by a map-based approach, and found that it encodes a CUC2-like NAC-domain protein previously reported as AtNAC1 (Takada et al., 10th International conference on Arabidopsis research). *CUC1* was expressed in the presumptive SAM during embryogenesis and at the boundaries of floral organs. Surprisingly, overexpression of *CUC1* was sufficient to induce adventitious shoots on the adaxial surface of cotyledons. *STM* and *KNAT1* were ectopically expressed in the cotyledons proceeding the adventitious shoot formation. These results suggest that *CUC1* acts upstream of the *STM* gene and regulates SAM formation during Arabidopsis embryogenesis.

496 Spiral phyllotaxis is maintained by a DNA topoisomerase I gene in Arabidopsis

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The genesis of phyllotaxis, which is often associated with the Fibonacci series of numbers, is an old unsolved puzzle in plant morphogenesis. We report here the characterization of a T-DNA insertion mutant of *Arabidopsis* that exhibits abnormal positioning of leaves and flowers instead of a regular spiral phyllotaxis. The mutant sporadically but with a high frequency produces multiple flowers from one node like umbelliferous plants and bifurcated shoots. Although the mutant flowers have a normal whorled structure of floral organs, the number of sepals and carpels is often increased and that of petals and stamens decreased. In addition, inflorescences and individual flowers of the mutant are spirally twisted and leaves show serrated morphology. The T-DNA insertion was found in a DNA topoisomerase I (*TOP1*) gene. The phenotype was completely rescued by a minimal construct containing a 1-kb promoter and a full-length cDNA of the wild-type gene. The functional significance of the *TOP1* gene in organ positioning will be discussed.

497 *RBE* encodes a SUP-like protein and regulates the petal development depending on the abaxial-adaxial axis

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Flowers have three types of spatial regulation systems. One is a whorl where organ identity genes are expressed. The others are abaxial-adaxial axis and lateral axis on which the organ primordia are aligned. Recent analyses of *Arabidopsis* flower mutants revealed the axis-dependent formation and growth of floral organs. The *rabbit ears (rbe)* mutant has defects in the petal development. The adaxial petals were deformed more frequently than the abaxial ones, suggesting that the petal development is regulated differently at the abaxial and the abaxial sides, and that *RBE* is involved in the process depending on the abaxial-adaxial axis. To investigate genetic interaction between *RBE* and other flower genes, we analyzed a couple of double mutants. A mutant of a class B gene, *ap3-5*, has petals transformed to sepaloid organs. The second whorl organs of *rbe ap3-5* double mutant were sepaloid and some of them were transformed to the abnormal shapes. This result suggests that function of *RBE* does not depend on the organ identity. However, in case of double mutant with a class A mutant, *ap1-17*, which has narrow petals and leaf-like sepals, almost all petals at both the adaxial and the abaxial sides were deformed. This result indicates close genetic interaction of *RBE* and *AP1* in petal development. We cloned *RBE* gene by map-based cloning methods. *RBE* encodes a SUPERMAN-like zinc finger protein, suggesting that RBE functions as a transcriptional factor. RT-PCR revealed that *RBE* was strongly expressed in inflorescence meristem and open flowers, but weakly expressed in siliques, seedlings and roots. We are now analyzing the *RBE* expression patterns in inflorescence by in situ hybridization.

498 A Screen for Factors that Regulate *APETALA3* Expression

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The *APETALA3 (AP3)* gene is a floral organ identity gene that is necessary for petal and stamen development in Arabidopsis. Work done in our lab has defined discrete regions of the *AP3* promoter required for different aspects of *AP3* expression. In particular, there is a 203 bp fragment that is required for both temporal and spatial regulation of *AP3* expression. Using the yeast one-hybrid system, we have identified at least one factor, 137, that binds to this region. 137 is a putative homeodomain-containing protein, and high levels of expression are only seen in inflorescences. *137* is expressed earlier than *AP3*, and the expression pattern of *137* and *AP3* largely overlap in the later stages of the flower (after stage 6). Progress in obtaining insertional mutations or transgenic loss-of-function lines of *137* will be presented. In addition, database searches have revealed that *137* belongs to a novel family of plant homeobox genes. Some of these family members are only expressed in inflorescence tissue. It is possible that some of these factors act in concert to regulate *AP3* expression.

499 *HALTED ROOT (HLR)* gene encoding 26S proteasome subunit 4 maintains root apical meristems.

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Root apical meristem (RAM) of primary root is established during embryogenesis. After germination, RAM controls root growth through balanced cell proliferation and differentiation. This RAM activity is maintained during continuous root growth. Although RAM is essential for root growth, little is known about this maintenance machinery. An *Arabidopsis* mutant *halted root (hlr)* shows aberrant post-embryonic root growth. Histological analysis revealed that normal cell layers in RAM of *hlr* formed in embryogenesis are disturbed at 2 days after germination, resulting root growth inhibition and root tip expansion. These data indicate that *hlr* undergoes normal embryogenesis, but fails to regulate cell division and differentiation in post-embryonic RAM. It suggests that the HLR protein is essential for the RAM maintenance machinery. Map-based cloning and complementation test revealed that HLR protein is a homologue of proteasome subunit 4 isolated from yeast. Proteasome is a huge complex to degrade poly-ubiquitinated proteins, which is known to work in various intracellular pathways such as cell cycle progression by cyclin degradation. Analysis using cyclin B::GUS, suggests that cell division activity in RAM of *hlr* decreases immediately after germination and is fully lost at about 20 days after germination. One of possible explanations for this result is; Reduction of proteasome function might cause cyclin accumulation and cell cycle arrest in RAM of *hlr*. These results indicate that *hlr* fails to maintain cell division activity in RAM after germination, suggesting that protein degradation pathway with proteasome containing HLR protein is essential for RAM maintenance. In *Arabidopsis*, we found a highly homologous gene of *HLR*, which shows 99% identity to HLR amino acid sequence. So we named this gene *HLR-LIKE PROTEIN (HLP)*. To confirm whether *HLR* and *HLP* gene have similar role or different function, we analyzed transcription of these genes. RNA gel blot analysis revealed *HLR* and *HLP* mRNA accumulation in all tissues. Now we are analyzing detail gene expression of both genes in RAM using reporter genes. Results of these experiments will be discussed.

500 The Arabidopsis *BREVIPEDICELLUS* gene is an important regulator of pedicel and internode development

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The overall above-ground architecture of flowering plants is determined to a large extent by the activity of key transcriptional regulators that control sets of genes involved in executing developmental programs. The *BREVIPEDICELLUS (bp)* mutant of *Arabidopsis thaliana* features compact floral internodes along with reduced pedicels with an altered attachment angle that results in downward-pointing siliques. This mutant has been used widely as a classical chromosome 4 marker, but there has been no detailed developmental or molecular analysis of bp plants reported to date. Analysis of the internodal regions of *bp* plants by scanning electron microscopy (SEM) revealed patchy regions of undifferentiated epidermal cells in the floral stem; microscopy of cross sections taken through the peduncle confirmed these observations and showed that the affected region extended into the 4-5 layers of subepidermal cortical cells. SEM analysis of the shortened pedicels of bp plants revealed an asymmetric effect on cell differentiation and elongation, with the abaxial side more strongly affected than the adaxial side. This asymmetry was further confirmed by anatomical analysis of cross sections through the pedicel and longitudinal sections through the floral nodes. These results established the developmental basis for the bp mutant phenotype. We cloned the BP gene and determined that it corresponds to the homeobox-containing gene *KNAT1* (Lincoln et al. Plant Cell 6: 1859, 1994). Southern blot and sequence analysis of the *KNAT1* locus from two alleles of bp revealed the molecular basis of the mutant phenotype. This work describes for the first time the developmental defects associated with the bp phenotype and clearly establishes a link between this phenotype and the previously characterized *KNAT1* gene.

BREVIPEDICELLUSbpBP

501 Polar auxin transport regulates organ formation at the periphery of the shoot apical meristem

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The entire shoot system is produced by shoot apical meristems (SAM). Although the precise sequence of events is not known, organ initiation starts with the isolation of a subset of cells from the meristem. This is followed by the specification of primordium identity and outgrowth. Moreover, organ primordia are produced in a regular pattern, called phyllotaxy. Although the signals controlling these early processes have not been identified, auxin has been associated with the regulation of shoot apex development. To explore the role of auxin in organ initiation, we have analysed the apex of the *pin-formed1* (*pin1*) mutant. PIN1 is a transmembrane protein involved in polar auxin transport and *pin1* mutants present a characteristic naked inflorescence stem. The dramatic reduction in polar auxin transport in the *pin1* mutant leads to major alterations in the process of organ initiation. In particular, our results show that cells at the periphery of the meristem express both markers of primordia and markers of primordia boundaries, demonstrating that in the mutant cells adopt a hybrid identity once they leave the meristem. Given the molecular nature of the PIN1 protein, we propose that localised polar auxin transport control the initiation of organ primordia by acting on cell identity at the shoot apical meristem. In order to study other regulators of primordia positioning, we have analysed the genetic interactions between PIN1 and three other factors involved in organ separation and initiation: CUP-SHAPED COTYLEDON(CUC)1/CUC2 and PINOID. Our results show that these four genes form a network controlling meristem formation and organ separation, thus highlighting the developmental importance of polar auxin transport.

502 Analysis of *FILAMENTOUS FLOWER* expression pattern using the GFP maker in *Arabidopsis*

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FILAMENTOUS FLOWER (*FIL*) gene encodes a zinc finger and an HMG-related domains. The expression of *FIL* gene is localized in abaxial regions of cotyledons, leaves and floral organs by *in situ* mRNA hybridization. In addition, the adaxial epidermal cells of rosette and cauline leaves of 35S::*FIL* plants partially changed into the abaxial epidermal cells. Therefore, *FIL* gene determines the abaxial identity of cotyledons, leaves and floral organs.(*Sawa et al*. Genes & Development, 13, 1079~1088, 1999, *Bowman et al*. Development, 126(18), 4117-28)
In order to analyze fine expression patterns of *FIL* gene in the development of lateral organs, we introduced the *FIL* promoter :: GFP fusion into wild-type *Arabidopsis*. In these transgenic plants, GFP signals are observed at abaxial surface of cotyledons, leaves and floral organs. The results confirmed our previous *in situ* pattern of *FIL*mRNA. Analysis of the transverse and longitudinal sections of the transgenic plants by confocal microscopy showed that GFP signals are detected in the two or three layers of cells at the abaxial side except cells at the vascular bundle.
The next question is how *FIL* gene gets the positional information based on the abaxial-adaxial axis. To obtain genetic answers against this question, we mutagenized the seeds of *FIL* promoter :: GFP transgenic plants with EMS and are screening the mutants with altered patterns of GFP signals.

503 Perturbation of cell division in developing embryos of *Arabidopsis thaliana*.

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Developing embryos of *Arabidopsis thaliana* undergo stereotyped patterns of cell division and expansion, leading to the regular cellular architecture observed in mature embryos. Presumably co-ordination of cell division and expansion between neighbouring cells must occur to ensure correct patterning and morphogenesis during development. We are perturbing embryonic cell division in *A. thaliana* to determine the nature and extent of intercellular communication between neighbouring cell types. A GAL4-GFP transactivation system is used to misexpress cell cycle regulatory genes in targeted cell types. The resulting perturbations are analysed using confocal microscopy and computer 3-D software to determine changes in the cellular patterning of the targeted cell types and their neighbouring tissues. Here we focus on the effects of altering cell division rates during embryonic development by misexpressing cell cycle regulatory genes in targeted cells of the vascular system and surrounding tissues of *A. thaliana*. We present data on the numbers, sizes and arrangements of cells in targeted and neighbouring tissues, and discuss the relationship between cell division, cell expansion and morphogenesis during embryonic development.

504 Screening for genes controlled by *SHOOT MERISTEMLESS* during meristem development

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The shoot apical meristem continuously provides new cells to build most of the above-ground parts of plants. The Arabidopsis *SHOOT MERISTEMLESS (STM)* gene keeps meristem cells undifferentiated and encodes a homeodomain-containing protein. To identify genes that mediate the effects of *STM* on cell division and differentiation, we initially generated plants in which *STM* function can be activated ectopically. These plants express a fusion between the STM protein and the steroid-binding domain of the rat glucocorticoid receptor (STM-GR); the fusion protein should be transported to the nucleus only after addition of steroid. In *stm* mutants expressing STM-GR, steroid treatment rescued meristem development, showing that STM-GR can replace *STM* function. In addition, ubiquitous activation of STM-GR disrupted leaf development and caused ectopic expression of meristem marker genes. To screen for genes subordinate to *STM*, we are using two approaches. The first is to characterise mutants that suppress the effects of ectopic *STM* activity (based on the expectation that the genes may also mediate *STM* function in the meristem). The second is to use cDNA arrays to monitor changes in gene expression after ectopic activation of *STM*. Progress on the characterization of STM-GR suppressors and candidate *STM* targets from cDNA array experiments will be presented.

505 An Enhancer of Glabra3 defines roles for bHLH proteins in all TTG1-dependent pathways

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We have shown that GLABRA3 (GL3) encodes a putative basic-Helix-Loop-Helix (bHLH) protein that interacts with the TTG1 protein. We also showed that overexpression of GL3 will suppress the trichome defect of the ttg1 mutation and here we show that this expression also suppresses defects in the other TTG1-dependent pathways. These pathways include: anthocyanin production, seed coat mucilage production, and position-dependent spacing of non-trichoblast (non-root hair) epidermal cells. However, mutations in GL3 only affect the trichome pathway and furthermore, GL3 has been considered to primarily control trichome branching. We performed a screen for enhancers of gl3-1, looking for totally bald plants. One new complementation group was identified that, when mutated, gives totally bald plants only in the gl3 mutant background. This group is shown to result from mutations in a second unlinked bHLH-encoding locus, which we call Enhancer of Glabra3 (EGL1). The double bHLH mutant, gl3/egl1, has a phenotype similar to the ttg1 mutation and is defective in all four pathways listed above. Using plant overexpression studies and yeast two-hybrid studies we show that EGL1, like GL3, interacts with other known regulators of TTG1-dependent pathways, and will form homodimers and heterodimers with GL3.

506 The role of DNA methylation in genomic imprinting in *Arabidopsis*

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Genes subject to genomic imprinting are differentially expressed depending on the sex of the parent they were inherited from. These alleles are epigentically modified during gameteogenesis so they are recognised as being of either maternal or paternal origin in the resulting progeny. The mechanisms by which these modifications are laid down are still being uncovered, although in mammals nearly all imprinted genes exhibit parent specific methylation patterns. In flowering plants accumulating evidence points to imprinting directly effecting the endosperm (and the embryo indirectly). If the normal ratio of genomes in the endosperm of *Arabidopsis* (2 maternal to 1 paternal genome) is manipulated by carrying out crosses between diploid and tetraploid plants the resulting seed show different and reciprocal phenotypes. Seeds inheriting extra maternal genomes (4x x 2x) show a low mature seed weight and an inhibition of mitosis in the endosperm, whilst those with extra paternal genomes (2x x 4x) have a high weight and endosperm overproliferation.

We have used these parent of origin effects to study the role of methylation in plant imprinting. By using hypomethylated transgenic plants in crosses with wild type plants we have shown that reducing methylation levels phenocopies the effect of adding extra genomes. This is consistent with a model in which hypomethylation of one parental genome prevents silencing of alleles that would normally only be active if inherited from the other parent. This suggests that methylation has an important role to play in parent-of-origin effects, and by inference parental imprinting, in plants. We are currently working on further defining this role by studying the expression of the Met1 methyltransferase using GUS promoter constructs.

507 A putative external loop domain in the 4th exon determines functional specificity of FT and TFL1

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FT (FLOWERING LOCUS T) is a floral inducer, which encodes a small protein showing homology to PEBP (phosphatidylethanolamine binding protein) and Raf kinase inhibitor (Kardailsky et al., Science 286: 1862 [1999]). *FT* overexpression causes early flowering while loss-of-function mutant flowers late, suggesting *FT* levels are critical in determining flowering time. Among 5 other genes with similarity to *FT* in *Arabidopsis*, the only other gene with a known role is *TFL1 (TERMINAL FLOWER 1)*, an inhibitor of flowering (Bradley et al., Science 275:80 [1997]). *FT* and *TFL1* share high homology but their action is opposite in terms of flowering time. Overexpression of *TFL1* leads to late flowering whereas *tfl1* loss-of-function mutants flower early. We and others have previously found that the relative levels between *FT* and *TFL1* are important for flowering time. Various exon swap constructs between *FT* and *TFL1* were generated and their effects on flowering time were investigated in an *ft-1 tfl1-1* double mutant background to determine their functional specificity. The results showed that the 4th exon of *FT* is important for its function. Further dissection of the 4th exon indicates that the region B in this exon distinguishes very between *FT*- and *TFL1*-like properties of a chimeric gene. Crystallographic structure of human PEBP and CEN (CENTRORADIALIS) from snapdragon (Serre et al., Structure 6: 1255 [1998]; Banfield and Brady, J. Mol. Biol. 297: 1159 [2000]) have shown that region B forms an external loop in PEBP and CEN. We speculate that the same is true for this region in TFL1 and FT, and that this region contacts FT and TFL1-specific interactors.

This work has been supported by fellowships from KOSEF and the Hoffmann Foundation (J.H.A.), and by a grant from USDA (D.W.).

508 Fluorescent *in situ* hybridization analysis of chromosome behavior in the Arabidopsis meiotic mutant, *sds*

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We previously reported the isolation of *sds* , which has a defect in meiosis of pollen mother cell (6th International Congress of Plant Molecular Biology). In wild-type meiotic cells, the homologues stay attached as bivalents until metaphase I and then separate into ten chromosomes at anaphase I. However, in the *sds* mutant, homologous chromosomes separate prematurely before metaphase I and ten chromosomes can be observed at diakinesis and metaphase I. The precocious separation of *sds* chromosomes result in abnormal distribution of chromosomes at anaphase I. In other words, instead of 5:5 even distribution as seen in the wild type, the distribution of *sds* chromosomes were 4:6, 3:7, and so on; sometimes some chromosomes did not move to either pole. As a result of this aberrant behavior of *sds* chromosomes, *sds* mutant produces more than four meiotic products, polyad, which are sterile. These results suggest that homologues might moved to the same pole sometimes. To further characterize the chromosome behavior, we conducted FISH analysis. At early prophase I (leptotene or zygotene stage), FISH signals could be observed as two points on the two homologous chromosomes, but at pachytene stage the signals seemed to merge into one spot in the wild type. During diplotene the signals separated from each other; then presumably due to condensation of chromosomes, the signals were again merged. At anaphase I the signals moved to opposite poles. In *sds* cells, the signals from homologous chromosomes never merged during prophase I and the signals moved sometimes to same direction and sometimes opposite directions. The results of FISH analysis indicate that in the *sds* cells, both homologues of chromosome I separated prematurely, and were distributed randomly to the two poles of the first meiosis. The results also suggest that the *sds* mutant might be abnormal even at the pachytene stage.

509 The Characterization of Seed Dormancy Genes in the Arabidopsis ecotype Cvi

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Research on seed dormancy in Arabidopsis in general is hampered by the relatively low level of dormancy in freshly harvested seeds in the commonly used laboratory strains Landsberg erecta (Ler) and Columbia. There are other accessions such as Cape Verde Islands (Cvi), which show a very strong dormancy. Cvi is used to analyse dormancy in Arabidopsis. To dissect genetically this quantitative trait, a set of recombinant inbred lines (RIL) derived from the cross Ler x Cvi has been used to detect and locate quantitative trait loci (QTL). This analysis revealed 7 loci, with genetic variation for seed dormancy. To characterize the individual loci, near isogenic lines (NILs) in a Ler background have been prepared. The germination behaviour of these NILs under different environments was analysed and hormone and inhibitor treatments were applied. Further more a QTL on chromosome 5 (DOG; Delay Of Germination) has been fine mapped. By combining DOG(cvi)alleles with some of the mutants known to be important in seed dormancy (e.g. ABA biosynthesis and seed maturation mutants) has given insight in the epistatic relationships between processes controlled by the various genes.

510 HEN1 promotes floral homeotic C function in Arabidopsis

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The floral homeotic C function is required for the specification of stamen and carpel identities in *Arabidopsis thaliana* . Severe loss-of-function mutations in the C function gene *AGAMOUS* (*AG*) lead to stamen-to-petal and carpel-to-sepal transformations in the flower. In addition, *ag* mutant flowers are indeterminate. Two other genes, *HUA1* and *HUA2* , are involved in all aspects of *AG* 's functions, although they mutate to weaker phenotypes. The *hua1-1 hua2-1* double mutant flowers display carpel-to-sepal transformation at the cellular level, i.e., some gynoecial valve cells exhibit sepal cell characteristics. In order to identify more components of the *AG* pathway, we performed an EMS screen in the *hua1-1 hua2-1* background and isolated mutations in several complementation groups that result in *ag* -like flowers. Two recessive alleles in the *HEN1* locus were found to enhance the *hua1-1 hua2-1* floral phenotype. Early flowers of the *hua1-1 hua2-1 hen1-1* or *hua1-1 hua2-1 hen1-2* genotype have sepals, petals, petals, and carpels from the outside to the inside of the flowers. Late flowers of the two genotypes also display phenotypes that indicate loss of carpel identity and floral determinacy. These phenotypes suggest that *HEN1* promotes C function. The *HEN1* gene was cloned by chromosome walking and encodes a novel protein.

511 Regulated overexpression of AGL15:effects on abscission and senescence in reproductive tissues

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The MADS domain factor AGL15 is preferentially expressed during embryogenesis, but may play multiple roles during plant development. Constitutive expression of AGL15 causes delays in the transition to flowering, delays in abscission and senescence in perianth organs, and delays in fruit and seed maturation. To better understand these effects, we have developed strategies to overexpress AGL15 only in subsets of cells or at particular times in development. When AGL15 is overexpressed in abscission zone cells using a chitinase promoter, no delays in abscission or effects on petal breakstrength are seen, indicating that action in the abscission zone cells is not sufficient to cause the observed floral effects. To test whether overexpression of AGL15 is sufficient to reverse or slow tissue senescence, AGL15 was expressed under the control of the SAG12 promoter. Vegetative and reproductive organs senesced at a rate that was indistinguishable from that of wild type. To express AGL15 in a controlled way at different times in development, we have developed a modified two component glucocorticoid-inducible system. This system, where the two components are combined by crossing, will be particularly useful in cases where low level expression may lead to embryo lethality or other detrimental effects on plant viability or reproduction. Funded by the UW-Madison Graduate School, USDA (96-35304-3699) and DOE/NSF/USDA Collaborative Program on Research in Plant Biology (DBI 96-02222).

512 HEN4 encodes a KH domain protein that regulates floral reproductive organ identities and plant size in Arabidopsis

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The floral organ identity C function gene *AGAMOUS* (AG) plays a key role in specifying the identities of the reproductive organs in *Arabidopsis*. Several genes have been reported to regulate the transcription of *AG*. However, little is known about whether there is regulation other than at the transcriptional level or how AG drives downstream target genes to establish the reproductive structures. *HUA1* and *HUA2* were isolated from an enhancer screen using the weak *ag-4* allele and shown to be new members of the *AG* pathway. A new screen in the weak *hua1-1 hua2-1* double mutant background has revealed further mutants with phenotypes indicating a comprised *AG* pathway. Two of these mutant lines contain recessive mutations in a novel locus, *HEN4 (HUA ENHANCER 4). hua1-1 hua2-1 hen4-1 and hua1-1 hua2-1 hen4-2* flowers exhibit defects in stamen and carpel identities and floral determinancy. Early arising flowers have petaloid stamens and enlarged gynoecia, later flowers show stamen-to-petal transformation and bear gynoecia with internal organs, and very late stage flowers resemble severe *ag* mutant flowers. In addition, the triple mutants are small in stature. This suggests that *HEN4* promotes C function and regulates plant size. We cloned *HEN4* with a map-based approach, and found that it produces two types of transcripts due to alternative splicing of the last intron. The two HEN4 protein variants contain either 4 or 5 hnRNP-K-homology (KH) domains, and belong to a family of at least 6 *Arabidopsis* KH domain proteins. KH domains are present in numerous proteins and thought to play very important roles in many cellular processes. Since the KH domain is a known RNA-binding motif, it is likely that *HEN4* regulates flower organ identity and plant size at the RNA level.

513 Composition and variation of the *Arabidopsis thaliana* pollen coat

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The extracellular protein/lipid matrix on the surface of *Arabidopsis* pollen is necessary for the initial steps of fertilization. Provision of a species recognition tag for the selective stigma cells is a key function of the pollen coat, but the molecule(s) responsible for recognition are not known. We have identified all of the major proteins in the pollen coat greater than 10 kd. Five of these proteins contain a lipid-binding oleosin domain and a C-terminal glycine-rich repetitive domain and correspond to genes clustered in a tandem array. To understand the function of these genes we are taking advantage of mutational analysis and natural variation. Though many genes in clusters degenerate to pseudogenes, the reading frames of all these genes are maintained in at least 5 ecotypes in the presence of numerous insertions/deletions suggesting that all are functional and necessary. Previous studies demonstrate that one of these, GRP-17, promotes efficient pollination. The similarity in gene structure suggests a high level of redundancy in these genes. To better understand the function of these genes we seek to identify a mutant with a deletion of this cluster. We investigated the variability of this gene cluster in the ecotypes Col, Ler and Cvi and found levels of polymorphism which are greater than average. Furthermore we found extensive divergence in the syntenic region of *Brassica oleracea*. Genes involved in species recognition on other systems have been shown to be highly variable, and the changes observed may promote speciation in plants.

514 The *WUSCHEL* gene has an essential function in ovule development

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The *WUSCHEL* (*WUS*) homeobox gene is expressed in the a small cell group, named organizing center, of shoot and floral meristems (Mayer *et al*., Cell 95, 805-815). Mutations in *WUS* result in misspecification of stem cells and premature termination of meristem activity (Laux *et al*., Development 122, 87-96). Misexpression experiments indicate that *WUS* is not only required but also sufficient to confer stem cell identity (Schoof *et al*., Cell 100, 635-644). *wus* mutants can initiate adventitious shoot meristems and can give rise to floral meristems. These floral meristems reiterate the mutant defect and terminate prematurely after the formation of the normal number of sepals and petals in a central stamen, without the formation of a gynoecium. Here we show that *WUS* is also expressed in the nucellus of ovules and address its function. Since no loss of function analysis was possible due to the lack of gynoecia in *wus* mutants, we provided *WUS* expression in shoot and floral meristems from a heterologous promoter that is not active in ovules. This allowed us to analyze ovules in a *wus* mutant background. Our results indicate that *WUS* plays an essential role in ovule development.

515 FUNCTION OF THE *BODENLOS* GENE DURING APICAL BASAL PATTERN FORMATION IN*ARABIDOPSIS*

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Apical-basal pattern formation in Arabidopsis embryogenesis involves a series of highly regulated cell divisions. A mutation in the *BODENLOS (BDL)* gene leads to a deviation from this division pattern resulting in a deletion of basal seedling organs. *bdl* seedlings are insensitive to 2,4 D treatment and the gene interacts genetically with *MONOPTEROS (MP)* and *AXR 1* suggesting that the *BDL* gene is involved in auxin signaling. The *BDL* gene encodes a putative auxin-response regulator which also interacts with *MP* in the yeast two-hybrid assay. In situ expression analysis and functional assays are currently under way in order to understand the effect of the mutation on pattern formation during plant embryogenesis.

516 The Effect of AGL 15 on Production of Somatic Embryos

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AGL15 (*AGAMOUS-Like 15*) encodes a MADS-box regulatory factor that is preferentially expressed during embryogenesis. AGL15 accumulates in a variety of embryos and embryonic tissues including zygotic, apomictic, somatic and microspore embryos, suggesting a role for AGL15 in global aspects of embryo development. To further test AGL15's role in regulation of development in an embryonic mode, we examined the influence of AGL15 on the production of somatic embryos. AGL15 levels were manipulated by the introduction of transgenes into Arabidopsis wild type and mutant backgrounds that produce somatic embryo tissue. The cauliflower mosaic virus 35S promoter driving expression of full-length *AGL15* (*MIKC* construct) provides increased and ectopic accumulation of AGL15 (Fernandez *et al*., 2000, Plant Cell **12**, 183-197). This same promoter was also used to drive expression of a form of *AGL15* lacking the C-terminal domain (*MIK* construct; seed provided by D. Fernandez, Univ. of Wisconsin). Similar constructs based on other MADS-box genes, *AGAMOUS* and *SRF*, produce dominant negative effects. We have found that cultured embryos with the *MIKC* construct can produce embryonic tissue in culture for extended periods of time. A recent report (Mordhorst *et al*., 1998, Genetics **149**, 549-563) of Arabidopsis mutants that can produce somatic embryos from enlarged shoot apical meristems (SAM) was intriguing to us because the very young SAM is one of the few places that we can detect AGL15 immunohistochemically after germination. We have introduced the *MIKC* and *MIK* transgenes into one of these mutants and have found that the *MIKC* construct in this background leads to production of somatic embryos from the meristem, whereas the *MIK* construct does not produce embryos. We are continuing work to test the effect of AGL15 levels on production of somatic embryos in other mutant backgrounds.

Supported by the University of Kentucky, Department of Agronomy, and by the National Science Foundation (IBN-9984274).

517 *SECRET AGENT* and *SPINDLY* have overlapping roles in plant development

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The *SECRET AGENT (SCA)* gene of arabidopsis encodes a protein with significant similarity to SPINDLY (SPY) which is a negative regulator of the gibberellin (GA) signal transduction pathway. To investigate the role of *SCA* in development and its possible functional similarities with *SPY*, two T-DNA insertional alleles of *SCA* were identified and used in genetic studies. While *sca* plants had no extreme morphological defects, chromosomes carrying linked mutant alleles of *sca* and *spy* genes had greatly reduced heritability. The genes are linked on chromosome III. Preliminary data suggested that there was reduced viability of both gametes and embyros inheriting chromosomes containing the linked *sca* and *spy* mutations. No double mutant plants were obtained. This data indicates a synthetic phenotype for *sca* and *spy* that is consistent with the model that the genes have overlapping functions in development. The results of a more detailed study of the inheritance of the linked *sca* and *spy* mutations will be presented.

518 *KOMPEITO* is required for exine formation and pollen-stigma adhesion in *Arabidopsis*

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In sexually reproductive plants, successive processes of cell-cell interaction between male and female reproductive organs are important events for fertilization. The first step is the pollen-stigma adhesion. Then pollen grains hydrate and pollen tubes grow toward the female gametophytes. However, little is known about the molecular mechanisms of these cell-cell interactions. To investigate these mechanisms, we screened mutants with defects of such interactions. We examined pollen grains and elongation pattern of pollen tubes in pistils of *Arabidopsis* plants using fluorescence microscopy after staining with aniline blue. Here we report a novel recessive mutant, *kompeito*. The shape of *kompeito* pollen grains is irregular and the fertility is reduced. We observed pollen grains using scanning electron microscopy. The surface of wild-type pollen grains shows a particular patterning characteristic of normal exine. *kompeito* pollen grains had irregular ridges instead of wild-type exine patterning. Through the development of pollen grains, we observed no obvious difference between wild type and *kompeito* before the formation of exine. Microspores develop normally. Once pollen tubes germinate following events proceed normally, suggesting that reduced fertility is caused by the defect of pollen adhesion to the stigma. This is the first genetic evidence that the exine is necessary for physical pollen adhesion to the stigma. We isolated *KOMPEITO* gene by chromosome walking. Predicted *KOMPEITO* gene encoded a putative trans-membrane protein of unknown function. Further analysis is in progress.

519 Unraveling the Function of Polycomb Proteins (FIE and MEA) in Plants.

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In flowering plants, two cells, the egg and the central cell nucleus are fertilized by two sperm cells in the haploid female gametophyte giving raise to the embryo and the endosperm tissue respectively. The endosperm is a tissue that supports embryo development. The *FERTILIZATION-INDPENDENT ENDOSPERM* (*FIE*) and *MEDEA* (*MEA*) genes encode WD and SET domain polycomb proteins, respectively. In mutant female gametophytes bearing a lesion in ether fie or mea, endosperm develops without fertilization. In contrast, when fertilization occurs, these mutations cause arrest of the embryo development. *fie* and *mea* mutations also cause parent-of-origins effects, whereby the wild type maternal allele is essential and the paternal allele is dispensable for seed viability. Recently we have shown that in Arabidopsis, similarly to insects and mammals, FIE and MEA proteins interact, suggesting that the molecular partnership of WD and SET domain polycomb proteins have been conserved during evolution. The overlapping expression patterns of *FIE* and *MEA* are consistent with a model whereby, in the female gametophyte FIE and MEA polycomb proteins function in a complex that suppresses gene transcription in the central cell thus controlling endosperm development until fertilization occurs, as well as controlling seed development after fertilization. Strategies allowing to understand the mechanisms by which these polycomb proteins function to regulate their down stream target genes during endosperm and embryo develop upon fertilization will be discussed.

520 Transcriptional activation by the Arabidopsis protein AINTEGUMENTA

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The floral development protein AINTEGUMENTA (ANT) is thought to function as a transcriptional regulator. It is a member of a large family of DNA binding proteins (AP2/EREBP family) that control plant growth and development in response to developmental or environmental signals. Transcriptional activation and repression activities have been demonstrated for several members of the EREBP subclass of these proteins. Using fusions between the GAL4 DNA binding domain and various parts of ANT, we have mapped the transcriptional activation domain of ANT to a 79 amino acid region in the amino terminal half of the protein. This region shows similarity to transcriptional activation domains in other proteins as it is rich in Ser, acidic, and bulky hydrophobic amino acids. In addition, we show that ANT can activate gene expression in both yeast and Arabidopsis through binding to a DNA sequence corresponding to an vitro determined ANT binding site.

521 Identification of transposon-tagged progamic phase genes in *Arabidopsis*

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The progamic phase of reproductive development involves post-pollination events from pollen germination to gamete fusion which are not easily accessible. As a strategy for the identification of progamic mutations which act gametophytically, we have used transposon insertional mutagenesis based on screening for distorted segregation of an antibiotic resistance marker. Screening of 3, 616 DS gene/enhancer trap lines allowed the identification of 19 potential gametophytic mutations. Cosegregation tests indicated that these transposon insertions are tightly linked to the reduced transmission phenotype. With the exception of one line, all lines showed a more severe reduction in genetic transmission of the transposon through the male than through the female gametes. Ten male-specific mutants produce pollen with normal cellular morphology, but fail to transmit the insertion during the progamic phase. No failed ovules or aborted seeds were observed suggesting no effect on fertilization events. Cloning of flanking genomic DNA at DS insertion sites revealed the identity of the disrupted genes. Three mutants, *pgp1*, *pgp2* and *pgp3*, potentially involved in cell signalling events, were selected for further investigation. *PGP1* codes for a putative plasma membrane protein containing a protein-protein interaction domain. PGP2 shows significant similarity with a sugar phosphate isomerase involved in capsule synthesis in bacteria. PGP2 could modify surface-associated polysaccharides and play a role in cell surface interactions. PGP3 presents significant similarity with RPT2 and NPH3, two signal transducers of the phototropic response (Sakai *et al*., Plant Cell 2000, 12:225-236; Motchoulski and Liscum, Science 1999, 286: 961-964). These proteins may function as adapter or scaffold proteins to bring together the enzymatic components of signalling pathways. Progress on genetic, phenotypic and functional analysis of these mutants will be presented.

522 *HUA1*, a regulator of stamen and carpel identities in *Arabidopsis*, codes for a nuclear, potential RNA-binding protein

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Stamen and carpel identities are specified by the combinatorial activities of several floral homeotic genes, *APETALA3*, *PISTILLATA*, *AGAMOUS(AG)*, *SEPALLATA1(SEP1)*, *SEPALLATA2(SEP2)* and *SEPALLATA3(SEP3)*, which all code for MADS domain DNA-binding proteins. *AG* and *SEP* genes also control floral determinacy. *HUA1* and *HUA2* were previously identified as regulators of stamen and carpel identities and floral determinacy because the recessive *hua1-1* or *hua2-1* allele affected these processes in plants with a lower dosage of functional *AG* (either homozygous for the weak *ag-4* allele or heterozygous for a strong *ag* allele, *ag-1*). *HUA2* was previously cloned and shown to code for a novel protein. Here we report the isolation of *HUA1* with a map-based approach. *HUA1* encodes a protein with six CCCH-type zinc finger motifs. This type of zinc fingers is also found in yeast, *C. elegans*, *Drosophila* and mammalian proteins. Several such proteins from mammals are known to bind RNA and play key regulatory roles in development. Here we show that HUA1 can bind ribohomopolymer in vitro, preferentially polyrG and polyrU at moderate salt concentrations. This suggests that, like other proteins with CCCH zinc fingers, HUA1 is a potential RNA-binding protein. Because HUA1 is localized in the nuclei of onion epidermal cells, it is likely that HUA1 is involved in RNA-related nuclear events such as splicing, export, degradation, etc. Although cellular target(s) of HUA1 remains to be determined, the RNA-binding property of HUA1 points to a new mechanism in the regulation of floral organ identites.

523 Spatial and temporal expression patterns of *APT1*, *2* and *3* in Arabidopsis floral tissue

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Adenine phosphoribosyltransferase (APT; EC 2.4.2.7) recycles adenine produced by the breakdown of nucleotides and nucleosides, to adenine monophosphate (AMP). There are five isoforms of APT encoded in the Arabidopsis genome, at least three of which are transcribed in floral organs based on northern analysis and RNase protection assays. Here we describe the use of in situ hybridization to localize these transcripts more precisely within floral tissues. Each *APT* had a dramatically different transcription pattern with respect to development and tissue abundance. *APT1* was constitutively transcribed but was induced strongly in the tapetum and pollen mother cells, prior to meiosis. *APT1* transcripts accumulated to the highest levels in these cells during meiosis and the formation of tetrads of haploid microspores. *APT2* transcripts were localized to the parenchyma cells around the xylem vessels mainly in the receptacle. *APT3* transcripts were detected in the ovary, particularly in the septum region (later also in the ovule), throughout microsporogenesis with higher levels detected after the tetrad stage. Levels of *APT2* and *APT3* increased in the *apt1-3* (APT1-deficient) mutant although their tissue expression patterns did not change. These results suggest that each APT isoform may have a distinct metabolic contribution based on its expression pattern. Moreover, APT2 and APT3 are likely unable to compensate for APT1 deficiency because of their different spatial expression patterns. Finally, *APT* transcript abundance increases in response to reduced APT activity. We are now investigating the possible metabolic signal(s) causing this change in *APT* transcript accumulation.

524 Isolation by cDNA-AFLP and characterization of a gene similar to bacterial Translocase I expressed during flower development of *Arabidopsis thaliana*

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The flower development is one of the most intriguing events during vegetal organogenesis. We are interested in the characterization of genes that are expressed in Arabidopsis thaliana late stage of flower bud development, wherein occurs the senescence phase of anther and the maturation of gynoecium tissues. We utilized **cDNA-AFLP** technique intending to isolate genes differentially expressed during this stage. We divided the flower development in 2 phases according to flower bud size and pollen development - phase 1: flower buds smaller than 1 mm and phase 2: flower buds bigger than 1 mm. Among the 20 putative transcript derived fragments (**TDFs**) specific from phase 2 wherein occurs the anther senescence, the fragment B2 is identical to the last exon of a putative gene (*T9A21_120*) that encodes a protein homologous to bacterial translocase I (UDP-N acetyl muramoyl pentapeptide transferase- **mraY**). In prokaryotes, this enzyme participates in peptidoglycan biosynthesis. After isolating its complete cDNA we visualized through Southern blot that *A. thaliana* Translocase I (**atTransI**) is a single copy gene. Northern blot analysis showed that this gene is expressed only in inflorescence. Furthermore, in situ hybridization experiments demonstrated that **atTransI** expression is restricted to microspores, anther tapetum and during ovule development. In order to elucidate the function of **atTransI** we will perform immunolocalization experiments as well as antissense construction in *A. thaliana*.

This work was supported by grants from the Brazilian Institutions FJUB, CAPES and CNPq.

525 Global Gene Expression Patterns in *emf1* Mutants

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The *embryonic flower*, *emf*, mutants skip vegetative development and form reproductive structures upon germination. Weak *emf* mutants, e.g., *emf1-1*, produce a small inflorescence and later a few flowers, while strong *emf* mutants, e.g., *emf1-2*, develop only carpelloid organs. Based on genetic and *AP1* expression studies, it was hypothesized that *emf1-2* begins life at a more advanced reproductive state than *emf1-1* (Chen et al., 1997). To study global gene expression patterns, we isolated RNA from wild type and two *emf1* mutants, *emf1-1* and *emf1-2*, and hybridized the RNA with Affymetrix GeneChips containing oligonucleotide probes of 8000 Arabidopsis genes (Zhu and Wang, 2000). Compared with wild-type seedlings, many flowering-related genes, especially flower-specific MADS box genes were more abundantly expressed in the seedlings of the two mutants. To confirm GeneChip data and to estimate RNA levels of low expressors, we carried out Reverse Transcription Polymerase Chain Reaction (RT-PCR) for *FT*, *TFL1*, *SOC1*, and *LFY* using RNA from wild type plants and *emf1* mutants at several developmental stages. We found that two flowering-time genes, *FT* and *TFL1*, which are highly expressed in the inflorescence meristems, were expressed at a higher level in *emf1-1*, but not in *emf1-2*, than in wild-type plants. These results indicate that the 1-2 week old *emf1-1* seedlings are at the inflorescence and flower phases, while *emf1-2* seedlings are at the flower phase.

We also investigated *EMF1* expressions in *co*, *ft*, and *emf1* mutants. *EMF1* expression levels in *co* and *ft* were similar to those of wild-type plants. However, *EMF1* RNA level was higher in *emf1* mutants, especially in *emf1-1*. This may be explained by *EMF1* self-regulation or the fact that *emf1* mutants contain flower meristems which accumulate higher levels of *EMF1* RNA. To confirm the expression level of *EMF1* in *emf1* mutants, we are introducing EMF1 promoter:GUS constructs to *emf1* mutants.

526 The Identifcation of Downstream Targets of the Arabidopsis Floral Development Protein AINTEGUMENTA

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The Arabidopsis protein AINTEGUMENTA (ANT) is a member of the plant-specific AP2/EREBP family of transcription factors. Members share either one or two copies of an approximately 70 amino acid domain called the AP2 repeat. Recently we determined the DNA binding specificity for ANT, which contains two copies of the AP2 repeat. This information is being used to identify potential targets of ANT regulation. A genome search for sequences with high similarity to the ANT consensus binding site has identified several such sequences that are found upstream of known or putative genes. We find that ANT binds to several of these sequences in vitro. Further characterization of these putative target genes using Northern blot analysis and RT-PCR in wild type, ant, and 35S::ANT backgrounds is currently underway. We will also present preliminary results from an alternative approach to identify ANT target genes which involves using microarray technology and a steroid-inducible form of ANT.

527 Gametophytic mutations affecting cytokinesis at pollen mitosis I

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To improve our understanding of molecular components and mechanisms involved in eukaryotic cytokinesis, two gametophytic cell division mutants were selected from independently generated EMS-muagenized pools. The mutants, named *two-in-one* (*tio*) produce ~35 % aberrant pollen grains containing either two free nuclei within the same cytoplasm, a single fusion nucleus, or collapsed pollen at the mature pollen stage. Reciprocal testcrosses revealed that genetic transmission of the *tio1* and *tio2* mutations was completely blocked through the male and very limited (~10 %) through the female. Developmental analysis during microgametogenesis revealed that the first aberrant phenotype is binucleate pollen, which results from the failure of cytokinesis at pollen mitosis I. Microspore nuclear migration and polarity are not disturbed in *tio* such that partial division occur at predicted asymmetric sites. In common with other sporophytic mutants such as *keule* , *knolle* , and *cyt1* , *tio* mutants produce incomplete dividing walls and internal wall stubs attached to the parental cell wall. Developmental defects were also observed during megagametogenesis by cytological analysis of cleared whole-mount ovules indicating a lack of cellularisation at the micropylar pole of the embryo sac. Molecular mapping of *tio1* and *tio2* showed that both mapped to the same location (72cM) on chromosome 1. Their similar phenotypic characteristics and map positions suggest that *tio1* and *tio2* are allelic mutations. Currently, molecular complementation of *tio1* is in progress. The molecular analysis of *TIO* would help to define its precise role in cytokinesis and provide valuable insight into the molecular mechanisms of gametophytic cytokinesis.

528 POP2, a gene required for guidance of Arabidopsis pollen tubes, is similar to class III omega aminotransferases.

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Successful fertilization in plants requires precise guidance of pollen tubes to the ovules; an event mediated by strong adhesion interactions and specific signaling events. Mutations in the pollen-pistil interaction gene, *POP2*, cause a specific and dramatic disruption of guidance: pollen tubes do not adhere to ovule tissue and fail to target the eggs. Crosses between mutant and normal plants show that this mutant is self-sterile: sterility occurs only when mutant pistils are pollinated by mutant pollen. To better understand the specific role of *POP2* in pollen tube guidance, we cloned the *POP2* gene by chromosome walking. A genomic fragment containing the cloned gene complements the *pop2* allele, restoring fertility to mutant plants, providing additional evidence that the cloned gene was indeed *POP2*. Sequence analysis of the cloned gene shows that *POP2* gene has strong homology to class III omega aminotransferases, enzymes that catalyze transfer of amino groups during the synthesis of omega amino acids. In addition to the *pop2-1* allele, we have also identified three different T-DNA insertional alleles of *POP2*. Our current work focuses on utilizing these *POP2* alleles to understand the function of *POP2* in pollen tube guidance by (i) identifying and characterizing the substrate for POP2 aminotransferase and (ii) identifying the genes through microarray experiments that show altered expression in the *pop2-1* mutant compared to the wild-type plants.

529 *otachi* enhances weak *ettin* mutants

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The gynoecium is the female reproductive structure of flowering plants. Because the *Arabidopsis* gynoecium is composed of several morphologically distinct tissues including apical stigma, style, ovary and basal stipe, it can be used as a model to study complex pattern formation. The phenotype of *ettin* (*ett*) mutants, as well as the expression pattern of *ETT* mRNA, suggests that *ETT* plays a crucial role in regional patterning of the gynoecium. An activation tagging modifier screen of the weak *ett* allele identified an enhancer named *otachi* (*ota*), with a phenotype strikingly similar to intermediate *ett* alleles. *OTACHI* was cloned and found to be a member of the Atrac/Rop family of GTPases. Preliminary characterization of this gene will be presented.

530 Enhancer trap lines YJ161 and YJ115 are expressed in the developing fruit

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The *Arabidopsis* fruit is a complex organ composed a several distinct tissues whose separate identities must be established during development. The valves, or seed pod walls, enclose and protect the developing seeds while the replum and septum divide the fruit into two halves. The valve margins attach the replum to the valves and form the dehiscence zone where the fruit opens to release the seeds when it matures. Several genes that are involved in the development of these tissues have been identified. The *SHATTERPROOF* (*SHP*) *MADS*-box genes redundantly regulate valve margin differentiation and later dehiscence of the fruit. Valve development after fertilization requires *FRUITFULL* (*FUL*), another *MADS*-box gene. *FUL* negatively regulates the *SHP* genes, limiting their expression to the valve margins. We have been examining enhancer trap lines to identify other genes involved in fruit development. The marker line YJ161 is expressed in the inner and outer epidermis of the valve margin. This expression pattern is different from those of the *SHP* genes which span the valve margin and may represent a developmentally distinct tissue type. The YJ161 valve margin expression is not regulated by *SHP*, but is negatively regulated by *FUL*. The YJ161 marker corresponds to the expression pattern of a putative zinc finger protein. Isolation of knockouts and overexpression of this gene are in progress to identify its role in fruit development. YJ115, another enhancer trap marker, is expressed in the abaxial replum and the valve margins and is regulated by the *SHP* genes. The T-DNA insertion in YJ115 is upstream of a gene encoding a protein of unknown function that contains a putative transmemebrane domain. Isolation of knockouts for this gene to determine its function is in progress.

531 Mis-expression of the Arabidopsis flowering time gene *CONSTANS*

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In many plant species, the transition to flowering is controlled by environmental signals such as day length. Arabidopsis is a facultative long day plant as it flowers earlier under long days (LD) than under short days (SD). In the *constans* (*co*) mutant, flowering is delayed under LD but not under SD. Previous studies have shown that *CO* is expressed throughout plant development, in both leaves and shoots. To address in which tissues *CO* expression is required to promote flowering, we are generating transgenic plants in which *CO* is expressed in specific tissues. *CO* expression patterns will then be analysed in relation to the flowering time phenotype. Two complementary strategies are being used to generate *CO* expression patterns. Both rely on a binary transactivation system that employs the synthetic transcription factor *LhG4*. First, we express *LhG4* in specific patterns from the promoters of the *UNUSUAL FLORAL ORGANS* (*UFO*), *PHANTASTICA* (*PHAN*), *CLAVATA1* (*CLV1*), *AINTEGUMENTA* (*ANT*) and *TobRB7* genes and then transactivate *CO* expression from an *LhG4*-responsive promoter. Secondly, we use an Ac/Ds transposon system-based enhancer trap strategy to generate a wider range of *LhG4* expression patterns.

532 Characterization of a male and female sterile *Arabidopsis* mutant

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We have isolated a T-DNA tagged *Arabidopsis* mutant that shows both male and female sterility with a high degree of penetrance. The mutation is fully recessive and sporophytic. The genomic sequence adjacent to the left border of the T-DNA was determined by TAIL-PCR. It suggests an insertion into the coding region of a predicted gene on chromosome one. Blast search revealed no homologues in *Arabidopsis* but 48% similarity to the rat TBPIP. This gene is suggested to play a role in male meiosis[1]. The homozygous mutant plant does not elongate its stamens and does not produce pollen. The female gametophytes of the homozygous mutant are able to attract pollen when the flowers are pollinated manually, suggesting that ovule development is normal. Embryos do not develop though. Complementation of the mutant is in progress. Further analysis of its phenotype is being carried out, aiming to identify the point of abortion in pollen development and the defect in female fertility.

1. T. Tanaka *et al*. (1997) Biochemical and Biophysical Research Communications 239, 176-181.

533 Characterization of Three Mutant Phenotypes Produced by Back-crossing a Single Reduced Fertile T-DNA Tagged *Arabidopsis* Line

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In a screening of the Feldman collection of T-DNA tagged *Arabidopsis* lines, a line that segregated in a 3:1 ratio of wild-type to normal size, fertility impaired individuals was identified. Preliminary characterization suggested that fertility impairment was most likely due to a meiotic defect, with the mutants producing primarily non-functional pollen of variable size and also exhibiting reduced female fertility. The line was 100% kanamycin resistant, indicating the presence of multiple T-DNA insertion points. Back-crossing of wild type pollen onto emasculated fertile individuals was performed to obtain a line containing a single insertion that co-segregated with the phenotype. Three groups of T-DNA tagged lines exhibiting different phenotypes resulted from these crosses, each of which appears to be segregating in a 3:1, WT to mutant ratio. In addition to the original phenotype, we have identified an unbranched fertile dwarf line and a highly branched fertility impaired dwarf line. Initial work with light microscopy and scanning electron microscopy indicates that the two lines of reduced fertile plants exhibit different mechanisms of fertility impairment. We have confirmed that the original line is a meiotic mutant, producing pollen of varying sizes. We suspect the impaired fertility of the highly branched dwarf line results from desiccation of developing pollen within the anthers. The epidermal layer of floral organs (sepals, petals, anthers and gynoecia) exhibit ruptured cells. Calyx development is abnormal causing the sepals to not fully enclose the developing flower. Anthesis is incomplete. Additional morphologic characters include broad, dentate basal and cauline leaves. The dwarf fertile phenotype appears to exhibit normal fertility and morphology, with the exception of having shortened racemes. The two dwarf conditions may have separate origin indicated by the difference in branching habit. All three mutant phenotypes can be maintained as heterozygous and homozygous lines. Hybrids of the three phenotypes were not observed.

534 PROMOTER TRAPPING OF SEVEN GENES EXPRESSED IN ARABIDOPSIS ENDOSPERM

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Recent studies in cereals and Arabidopsis have revealed a conserved developmental pathway for endosperm development in which cellularization of the endosperm coenocyte is initiated by establishment of nucleo-cytoplasmic domains by a nuclear based radial system of microtubuli. Cellularization is mediated by cytoplasmic phragmoplasts depositing anticlinal cell walls in the interzones between neighboring arrays of nulear based radial microtubuli. To further elucidate endosperm development in Arabidopsis we have initiated a screen for endosperm-expressed genes using a collection of promoter-trap lines. The lines were generated by root transformation with pMHA2 vector (promoterless gusA gene at the right T-DNA border). Among 309 independent lines examined, 37 lines (12%) exhibited GUS activity in seeds. Of these, 10 lines (27%) displayed GUS activity both in endosperm and embryos, 13 lines (35%) in embryos only, 12 lines (33%) in three or more seed organs and 2 lines (7%) in integuments. In total 22 lines (60%) exhibited GUS activity in endosperm and other organs but none of the lines showed GUS activity only in endosperm. We decribe seven lines in this poster (A-G). Two of them display stage-dependent GUS activity in the endosperm only (lines A and B). In line A GUS activity is localized in chalazal endosperm at the heart stage of development. Using LR-iPCR we isolated T-DNA flanking regions. We detected integration in the upstream region of a gene with unknown function. In line B GUS activity is in both endosperm and embryo. At the heart stage of development, a portion of seeds from this line show GUS activity only in the endosperm. GUS stained endosperm nuclei are grouped around the heart stage embryo and vary in size. We interpret this phenotype to indicate that nuclear divisions in the endosperm are unsynchronized. Sequence data revealed the integration of a promoterless GUS expression cassette in the upstream regions of a gene with unknown function. Sequences from 5 additional lines (C-G) showing GUS activity in the endosperm and embryos have been obtained. In two of them we detected integration in upstream regions of known genes, but with hitherto undescribed patterns of expression.

535 PROMOTER TRAPPING OF THREE GENES EXPRESSED IN ARABIDOPSIS SILIQUES

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In a screen of Arabidopsis promoter trap lines (Vector pMHA2- promoterless gusA gene at the right T-DNA border; Abul Mandal, University of Skövde, Sweden) for endosperm genes (poster by B. Stangeland et al.), we detected three lines displaying GUS staining in 1) **stomata**, 2) **embryo and hydatodes** and 3) **seed coat**. Among the first 309 lines examined, 50 lines (16%) showed GUS expression in siliques. Of these, 22 lines (44%) displayed GUS expression only in seeds, 9 lines (18%) in silique tissue only and 4 lines (8%) exclusively in the siliques abscission zone. Twelve lines (24%) showed GUS staining both in siliques and seeds and 3 lines (6%) exhibited GUS activity in both seeds and in the abscission zone. Based on Southern blot analysis, approximately 50% of the lines posses single T-DNA copy insertions. **Stomata** -One of the transgenic lines shows GUS activity in guard cells on siliques and stem but not (or very weakly) on leaves. GUS staining is visible also in roots and auxillary meristems. Using Long Range Inverse PCR we isolated T-DNA flanking regions from this line. Sequence data revealed the integration of a promoterless GUS expression cassette in a gene with an unknown function. **Hydatodes** (leaves) **and embryos** -We also analyzed a line with the GUS activity in the hydatodes and embryos. Sequence analysis of the isolated plant flanking region revealed integration in the gene coding for a nitrate chlorate transporter protein with an unknown expression pattern. **Seed coat** -Two of our lines showed GUS activity only in the seed coat. We sequenced isolated plant promoter sequences from one of these lines and confirmed integration in a gene with an unknown function. We are currently working on several lines showing GUS activity in embryos and transport system of the seed (maternal tissue, funiculus and embryo suspensor).

536 New roles of COP9 signalosome in Arabidopsis development revealed by partially functional mutants of CSN1

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The COP9 signalosome (CSN) has been genetically defined as a repressor of photomorphogenesis in Arabidopsis seedling development. Very little is known about its function in other developmental processes and the specific role of its 8 subunits. Here, we have generated partially functional COP9 signalosomes by expressing truncated forms of the CSN1 subunit in a CSN1 null mutant of Arabidopsis (*fus6-1*) via stable transformation. We show that assembly of the complex requires the middle region or the complete C-terminal half of CSN1, while the N-terminus of CSN1 is essential for the function of the complex. The mutant COP9 signalosome lacking the CSN1 N-terminus can not fully rescue the mutant but confers a phenotype that is distinguishable from *fus6*. The complex formed with a C-terminal deleted CSN1 can fully rescue the seedling defects of fus6 but showed impaired ability to produce high levels of the complex. Consequently, this partial loss-of-function mutant (*fus6/A1*) exhibits specific phenotypes in flower development and in inflorescence apex, demonstrating critical physiological functions of the COP9 signalosome beyond photomorphogenesis and seedling development. And one CSN1 full lenth transgenic line which has low level COP9 signalosome showed the similar phenotypes to *fus6/A1*. Key Words: COP9 signalosome / CSN1-FUS6 / flower development / inflorescence / protein complex

537 *HEN2* encodes a putative DExH box helicase involved in the specification of reproductive organs

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Reproductive organ identity in *Arabidopsis* is regulated through the activity of Class B, C and E genes. Class C and E genes are required for the production of carpels, while the additional presence of Class B genes leads to stamens in the third whorl. *hua enhancer2-1 (hen2-1)* was identified through its enhancement of the double mutant between the weak Class C genes *HUA1* and *HUA2*. Triple mutant *hen2-1 hua1-1 hua2-1* flowers have mosaic sepal/petal/stamen organs in the place of stamens, and sepal-carpels rather than carpels in the fourth whorl. Single *hen2-1* mutants and double mutants with either *hua1-1* or *hua2-1* also show defects in the fourth whorl. These results suggest that *HEN2* is required for proper specification of the reproductive organs, by influencing the activity of both Class B and C genes or of Class E genes. *HEN2* maps near the centromere of chromosome 2 and was cloned through chromosome walking. Comparison to GenBank sequences suggests that *HEN2* encodes a putative RNA helicase containing a DExH box. HEN2 is highly similar to the yeast helicases Ski2p and Dob1p which are associated with the exosome, a complex of RNases which acts in RNA processing and degradation. This implies that post-transcriptional processes play specific roles in organ identity specification.

538 *GIGANTEA,* an Arabidopsis gene that controls circadian rhythms and flowering time

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In plants, flowering occurs through a combination of endogenous and environmental signals. One of the main environmental signals which contributes to the transition to flowering is photoperiod. Measurement of the daily light/dark cycle involves the circadian clock, a self-sustaining timekeeper found in plants, microorganisms and animals. Once the photoperiod is recognised as being inductive, flowering occurs. Mutations have been isolated in Arabidopsis that delay flowering under inductive photoperiods. These genes are assigned to the long day flowering pathway, and include the gene *GIGANTEA* (*GI*). *GI* mutants show delayed flowering under LD conditions. In addition to the effect on flowering time, mutations in *GI* have additional effects, such as altered circadian rhythms of clock regulated genes, disrupted phytochrome light signal transduction, and resistance to the herbicide Paraquat. *GI* is cloned and encodes a clock regulated, nuclear protein of 1173 amino acids. *GI* mRNA abundance is circadian clock regulated and peaks 8-10 hours after dawn. The timing and duration of this peak is influenced by daylength. The effect of *GI* mutations on clock regulated genes indicates that GI has a role in maintaining the circadian expression of these genes, in addition to its role in the photoperiodic control of flowering. We have studied the role of GI in both processes by mis-expressing *GI* from heterologous promoters, and these data will be presented.

539 A Knockout for Every Gene and a Chip for Every Purpose

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A progress report will be provided on recent developments with two genomic technologies developed at the University of Wisconsin-saturation reverse genetics using a collection of insertionally mutagenized 'knockout' Arabidopsis plants, and a maskless array synthesizer (MAS), for producing high density DNA oligonucleotide arrays 'on the fly'. Our reverse genetic approach involves the rapid screening of several hundred thousand insertionally mutagenized Arabidopsis lines, for the isolation of knockout plants for any gene of interest. We have previously reported on a mathematical treatment required to isolate a knockout in any gene of interest (Krysan et al., 1996) and now report empirical observations on experimental progress towards obtaining a knockout for each and every one of the 26,000 Arabidopsis genes. Progress towards the establishment of a computer database for T-DNA insertions, using TAIL PCR to generate flanking sequences, will also be described.

As a general tool for genotyping and for discerning the effects of a particular mutation on global genome expression, we have been testing existing and new technologies based on high density oligonucleotide arrays.

Recent experiments utilizing a maskless array synthesizer (MAS) (Singh-Gasson et al., 1999) which is capable of generating, in only four hours, custom chips containing a half million different oligonucleotides on a 2 cm squared glass surface, will be described. The MAS uses a digital micromirror device developed by Texas Instruments, to generate virtual masks for use in photolithography and is a powerful tool for bringing combinatorial chemistry to the benchtop in research laboratories.

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Singh-Gasson, S., Green, R.D,, Yue, Y., Nelson, C., Blattner, F., Sussman, M.R. and Cerrina, F. 1999. Maskless fabrication of light-directed oligonucleotide microarrays using a digital micromirror array. Nature Biotechnology 17:974-978.