1-01 Functional proteomics of plastids from *Arabidopsis thaliana* through prediction and experimentation

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Each plant cell contains plastids, which are essential organelles containing up to 3500 proteins. Plastids vary in size, shape, and function and differentiate from meristem-localized proplastids and are responsible for synthesis of key molecules required for the architecture and functions of plant cells. To define different plastid (sub)proteomes and characterize a number of key plastid localized processes, we are using a combination of reverse genetics and proteomics, including mass spectrometry and bioinformatics. We are systematically identifying the proteome of chloroplasts and their protein-protein interactions using different subcellular fractionation and protein separation techniques followed by mass spectrometry (MALDI-TOF MS and on-line and off-line nano-ESI-MS/MS). The experimental proteome data are used for training neural network subcellular protein localization predictors to screen the Arabidopsis genome for the complete plastid proteome. The mass spectrometry data are also used to correct gene annotation errors and determine e.g. alternative slicing and post-translational modifications. We will also report on the identification of a chloroplast localized 350 kDa Clp core protease complex in Arabidopsis composed of 11 different Clp gene products. This protease complex is likely to be involved in subcellular protein house-keeping, as well as regulation of plastid gene expression. We have recently identified several disruption mutants in different Clp genes. These lines are used for proteomics studies aimed at determining the effect of Clp gene disruption on the Clp complex composition, as well as plastid protein expression patterns and identification of Clp substrates in plastids.

1-02 Investigation of the *Arabidopsis thaliana* interactome using an automated 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 thaliana* in view of the availability of the complete nuclear genome sequence in 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. With this method, pre- and counter-selection for excluding false positives are combined with robot arraying technology for handling large numbers of yeast clones. Bait proteins are screened against a GAL4-activation domain fusion Arabidopsis cDNA library. The identity of the interacting proteins expressed in individual yeast clones is determined by PCR, restriction, and sequence analysis of isolated plasmids. We use our automated high throughput yeast 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.

1-03 High-resolution SNP markers for QTL mapping in *Arabidopsis thaliana*

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High-density markers are vital for the comprehensive analysis of complex traits involving polygenic loci, and for association studies between genotypes and phenotypes. With the completion of the sequencing of the Col-0 ecotype of *Arabidopsis thaliana*, the Arabidopsis SNP discovery project aims to generate a genome-wide high density SNP marker set for the plant model system, *A. thaliana*. The SNPs are being identified by an automated sequence alignment and SNP calling program developed at SGTC, using sequences from PCR amplicons spaced at 30 kb intervals from Col-0, Ler-0, and Cvi-0 ecotypes. Markers at 100 kb intervals are being validated either by pyrosequencing or the Taqman SNP detection assay, and will be used to genotype 94 naturally occurring ecotypes of *A. thaliana*. In addition, we are collaborating with Affymetrix on a resequencing chip that will be used to resequence 200 bp fragments from chromosome III at 60 kb intervals using both PCR amplified DNA probes and labeled genomic DNA. Currently we have identified SNPs at an 80 kb resolution, and progress on the genotyping and Linkage Disequilibrium analysis will be reported.

1-04 Arabidopsis gene regulatory network revealed by whole genome expression analysis

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Genome-wide expression analyses not only identify genes potentially involved in biological processes, but also provide opportunities to reveal gene regulation mechanisms. In order to characterize the transcription regulation network in Arabidopsis, we analyzed the transcription pattern of 402 genes encoding stress responsive transcription factors in 80 samples collected at different developmental stages in various genetic conditions and stress environments. Based on their expression specificity, their potential functions and roles in the common regulatory network were assigned. Some of them are likely to be important in controlling downstream gene expression in signal transduction pathways, since their activation following bacterial pathogen infection was significantly reduced or abolished in the mutants that have defects in salicylic acid (SA), jasmonic acid (JA) and ethylene signaling. Promoter sequence analysis of the co-expressed target genes not only confirmed the importance of known ABRE and DRE elements but also revealed the new cis-acting elements. To understand the effect of chromosomal location on the transcription regulation, a transcriptome map has been developed based on over 250 transcriptome profiles. These profiles were generated using the Arabidopsis whole genome GeneChip microarray with probe sets for 26,000 genes. Preliminary results identified enriched expression chromosomal domains for normal development. The existence of these domains is further supported by the bootstrap analyses. Regions of increased gene expression (RIDGEs) containing genes encoding structural proteins, chlorophyll A-B binding protein, ribosomal RNA were also identified. Our results established a global framework for further understanding the gene regulatory network in this important species.

1-05 REGIA, an EU project on functional genomics of transcription factors from *Arabidopsis thaliana*

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Transcription factors (TFs) are regulatory proteins that have played a pivotal role in the evolution of eucaryotes and that also have great biotechnological potential. REGIA (REgulatory Gene Initiative in Arabidopsis) is an EU-funded project involving 29 European laboratories with the objective of determining the function of virtually all transcription factors from the model plant, *Arabidopsis thaliana*. REGIA involves: i) the definition of TF gene expression patterns in Arabidopsis; ii) the identification of mutations at TF loci; iii) the ectopic expression of TFs (or derivatives) in Arabidopsis and in crop plants; iv) phenotypic analysis of the mutants and mis-expression lines, including both RNA and metabolic profiling; v) the systematic analysis of interactions between TFs; and vi) the generation of a bioinformatics infrastructure to access and integrate all this information. In this communication we will present the progress on these different activities In summary, we have already defined many transcription factor families through bioinformatic analysis, the TF gene array has already been prepared and is being used by the different groups for expression analysis under 49 defined conditions/treatments. More than 175 transcription factor mutants have been isolated, and more than 250 TF-derived constructs have been prepared and introduced into transgenic plants which are currently being analysed. Metabolic profiling techniques have been set up. In addition, more than 800 full-size TF ORFs have been cloned in yeast two-hybrid vectors and information concerning interactions among TFs is starting to emerge.

1-06 A sequence-indexed library of insertion mutations in the Arabidopsis genome

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The functional characterization of the over 25,000 genes uncovered by the Arabidopsis genome sequencing project will require a large multidisciplinary effort. An indispensable element in reaching this goal is the availability of knockout mutants for each one of the Arabidopsis genes. We have generated a TDNA collection consisting of more than 150,000 individual mutant plants. Systematic sequencing of the insertion sites in these lines is being carried out at The Salk Institute Genome Analysis Laboratory. Thus far, more than 40,000 TDNA locations corresponding to more than 30% of the predicted genes have already been determined. Sequence data are made available with daily updates via a web-accessible graphical interface, the Arabidopsis Gene Mapping Tool (http://signal.salk.edu/cgi-bin/tdnaexpress). All DNA sequences are deposited into GenBank and provided to The Arabidopsis Information Resource, whereas seeds from the respective T-DNA insertion lines are donated to the Arabidopsis Biological Resource Center. The analysis of the distribution and structure of the large number of insertion sites determined in this project is providing crucial information about the biology of the Agrobacterium-mediated plant transformation.

1-07 Chloroplast functional genomics

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We study photosynthesis and its interconnection with other plastid and extraplastid functions in A. thaliana. Molecular and physiological functions of photosynthesis-relevant genes involved in diverse cellular functions are dissected by analysing mutants with altered quantum yield. In addition, mutants for almost all nuclear encoded photosystem I subunits were obtained by reverse genetics and characterised. All 4000 genes coding for chloroplast proteins were identified bioinformatically, and PCR-amplified gene-sequence-tags of such genes immobilized on nylon filters are used for organelle-wide expression studies to classify and characterise chloroplast-function mutants, as well as the plant response to environmental stimuli.

1-08 Differential RNA expression of core cell cycle genes during cell cycle re-entry using synchronised Arabidopsis cell line MM1

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Dispersed plant suspension cultures offer the possibility to study cell division in the absence of developmental processes and represent a useful system to investigate the cell cycle for basic and applied research. Their particular value is that they provide a homogeneous population of nearly identical cells that offer material for synchronisation by various procedures. Synchronised suspension cultures are powerful tools in plant cell cycle studies and essential for the discovery and analysis of cell cycle controlling genes. To identify different timing of expression of genes, particularly at the transition from G0/G1 into S-phase, samples of partial synchronised fast growing Arabidopsis cell line MM1 were used for differential RNA analysis. Here we describe the bioinformatic analysis of the data obtained after Massively Parallel Signature Sequencing (MPSS) of the first plant tissue samples to globally analyse the transcript profiles. We show that CDK-related protein kinases in Arabidopsis show differential expression timing during cell cycle progression and we further demonstrate that the CDK-inhibitor gene ICK2 (KRP2) shows a distinct expression pattern during cell cycle re-entry. Synchronous Arabidopsis cell suspensions represent a potentially useful system together with MPSS as an analytical tool to observe strikingly different expression of core cell cycle genes during cell cycle re-entry and in particular for the discovery of new cell cycle related genes.

1-09 Discovery of gene-function relationships with knock-out Arabidopsis plants

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During the last 5 years ZIGIA (Centre for functional genomics in Arabidopsis) has provided the scientific community with insertion-tagged mutants of *Arabidopsis thaliana* Col-0 as the basis for gene-function relationship studies. ZIGIA currently uses a population of 16,000 Arabidopsis single-seed descent (SSD) lines, each carrying 1-20 copies (6 independent insertions on average) randomly distributed insertions of the maize transposon En/Spm. These 100,000 insertions are estimated to result in at least one insertion per gene. Another resource used, is a mutant collection of several thousand T-DNA activation-tagged lines. The main results were analyzed to get some answers to frequently asked questions. For example, although tagging with En/Spm or with T-DNA does result in different pros and cons, which were hotly debated at the start of the project, each approach has contributed significantly to the advancement of our knowledge. From the 26 gene-phenotype relationships that we discovered so far, 10 were discovered with transposon tagged lines and 16 were obtained with T-DNA tagged lines. Similar discussions were held about the best strategy for screening, either by forward screening (phenotype-to-genotype) or by reverse genetics (genotype-to-phenotype). The facts are that forward screening resulted in 11 publications and reverse genetics in also 11 publications. Clearly the power of these techniques is best exploited by combining them. We currently concentrate on tools and techniques to do this more efficiently.

1-10 The use of genomic tools to study the regulation of leaf senescence in Arabidopsis

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Understanding the mechanisms by which plants control senescence is of great economic importance and may enable crop breeders to generate improved varieties for traits such as yield, nutrient quality and shelf life. Leaf senescence is a programmed event responding to external and internal signals and is controlled in a highly co-ordinated manner. Senescence requires de novo gene expression and protein synthesis and many genes have been isolated that show enhanced expression during this terminal phase of development. Many of the genes identified so far are involved in downstream events such as protein and lipid breakdown pathways but little is known about the regulatory signals and pathways that control senescence. Using various techniques, such as subtractive hybridisation and cDNA AFLP, we have isolated several genes encoding putative regulatory factors that show enhanced expression during senescence. These genes appear to be involved in different signalling pathways leading to gene expression during senescence. To establish the role of these regulatory factors during developmental senescence we are currently carrying out a functional analysis using Arabidopsis insertion mutants and both senescence specific microarrays and Affymetrix arrays. In addition, we are studying the role of known signalling pathways in controlling gene expression during senescence using mutants defective in these pathways.

1-11 RNA quality control experiments for cDNA microarray analysis

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Microarrays assay differential gene expression by co-hybridisation of fluorescently labelled probes prepared from different RNA sources. As with many other RNA-based assays, the purity and quality of the starting RNA has a significant effect on the results of the assay. This study highlights necessary RNA quality control experiments before probes can be labelled and DNA microarray analysis performed. RNA was isolated from a control, *Arabidopsis thaliana* ecotype Columbia plants, and a novel mutant with enhanced disease resistance. PCR studies were undertaken to determine whether RNA samples were contaminated with DNA, which could produce false positive results in cDNA microarray analysis. Samples with DNA contamination were subjected to DNaseI treatment, and the reaction was cleaned up either by means of phenol chloroform extraction, or by purifying the RNA on a spin column. Thereafter, RNA integrity was assessed by electrophoresis of the RNA on a formaldehyde agarose gel. The RNA was blotted onto a nylon membrane, and subjected to Northern analysis using a constitutively expressed gene (RUBISCO-binding protein) as a probe. Clear hybridization signals confirmed that the mRNA was intact. This RNA will be labelled with Cy dyes during the cDNA synthesis step, and then hybridized to cDNA microarrays in a collaborative effort aimed at establishing Microarray technology in South Africa. Several hundred pathogen response signalling cDNAs from Arabidopsis will be arrayed on glass slides both at the University of Pretoria, using a Generation III ArraySpotter (Molecular Dynamics) and at the University of Cape Town, which is equipped with a BioRobotics MGII TAS arrayer. These joint experiments will assist in establishing experimental skills and appropriate microarray data analysis software in each lab.

1-12 From genetics to digital modelling, and back: The *ltd* mutants impaired in the modulation of SA-pathway by phytochrome signaling

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The represention of cellular signal transduction has been examined in the light of the information theory. We show that the "crosstalk" phenomenon that connects signaling pathways at the level of cell membrane, cytoplasma, or promoters (through coaction of cis-regulatory elements in, for instance, enhanceosome), can be described by sets of logical operations performed by Boolean gates. In addition, the Boolean (aka digital, or numeric) modelling allows simulation of the cell signaling on a computer. These simulations nicely mimick the activity of any regulating network and provide an efficient tool to represent the effect of simple and multiple mutations in a cell; they also allow the deduction or detection of missing or additional regulatory elements with their potential location in a regulatory circuit. To introduce the digital formalism, we present the rules of translation to convert genetic and microarray data into Boolean language, with examples selected from a) the regulation of the pathogenesis-related genes (*PR*) in Arabidopsis, b) the light signal transduction pathway controlling far-red-light regulated genes, and c) from the phytochrome-regulated pathway modulating the *PR* expression downstream of SA. The *ltd* (for light-to-defense) mutants and their respective responses to light and SA will be used as an example to illustrate the basic concepts for the identification of mutants affected in interfering pathways.

1-13 Identification of genes involved in gametophytic process of spore formation, pollination and fertilization in Arabidopsis

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Formation of haploid spores marks the initiation of haploid phase of life cycle in seed plants, and the process of pollination-fertilization is the transmit connection from haploid phase to diploid phase. So far, very little has been known about the mechanisms involved in gametophytic processes of spore formation, pollination and fertilization. The main difficult is due to the limitation of technologies available for effectively identifying genes involved in the processes. We reported here our attempt to identify genes involved in the processes of spore formation, pollination and fertilization using gene-trap technology (Sundaresan et al 1995, Gen & Dev 9: 1797-1810), that allows identifying the semi-fertile gametophytic mutants effectively. So far, we have obtained more than 300 mutants showing gametophytic defects, which includes three types of mutations: defects in male parts only, defects in female parts only and defects in both male and female parts.

1-14 Macrosynteny analysis between *Cucumis melo* map and *Arabidopsis thaliana* genome, and microsynteny in a region containing a resistance gene cluster.

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Macrosynteny studies are carried out on whole genome level, by comparing genetic maps, whereas microsynteny questions the degree of genetic order conservation on a more local scale. Melon (*Cucumis melo* L.) is a vegetable crop, its genome has been estimated in 450 Mb and its genetic map, constructed essentially with RFLPs and SSRs, covers a total genetic distance of 1197 cM with an average density of 3,1 cM/marker. As a counterpart in the comparative analysis *Arabidopsis thaliana* was chosen. In silico analysis of the 204 melon RFLP markers with unique and known location in its genetic map against the Arabidopsis data bank using TBLASTX program allowed to locate significantly similar sequences in Arabidopsis genome. The computing protocol SHOWMAP-FITMAPS allowed to construct the comparative maps. Synteny was detected between markers grouped in melon linkage groups and Arabidopsis chromosomes, without conserved order between them: 28 groups of linked markers in Arabidopsis corresponded to 21 in melon. The melon resistance gene homologues MRGH63 and MRGH4 have been mapped and localised in Linkage Group 4. A contig of BACs in this region was created and the complete sequence of one BAC from the contig allowed to determine the genes present in this region. The sequence analysis detected microsynteny between two regions of Arabidopsis with similar genes in the same order and orientation as in melon region. With the present work we will try to gain knowledge on the melon genome making the most of the advances achieved with Arabidopsis and other genomes.

1-15 Arabidopsis GeneChip™ microarrays: A useful tool for novel promoter discovery.

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Microarray analysis of gene expression is a robust method to monitor global changes in gene transcription in response to biotic and abiotic stresses as well as developmental cues. Microarray data has been useful in inferring the function of novel genes based upon their expression profiles. Genes that show co-regulation with known genes in response to developmental or environmental signals are likely to have a role in the process under investigation and share common regulatory elements. Recently we have carried out microarray experiments designed to uncover genes that are expressed constitutively in all organs at various developmental stages or in specific organs and/or developmental stages. We have identified candidate promoters based upon the expression profiles of the associated transcripts. Promoter candidates were obtained by PCR and fused to a GUS reporter gene containing an intron. Both histochemical and fluorometric GUS assays have been carried out on stably transformed Arabidopsis plants and GUS activity has been detected in the transformants. Further, transient assays with the promoter::GUS constructs have been carried out in tomato leaf disks and GUS activity has been detected by histochemical staining. Thus, our results indicate that DNA arrays not only provide a useful method for the systematic evaluation of quantitative and qualitative changes in gene expression and gene function analysis, but also provide an extremely powerful tool for discovery of promoters that can be used to regulate genes of interest in transgenic crops.

1-16 Using cauliflower to find conserved non-coding sequences in Arabidopsis

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Conserved non-coding DNA sequences obtained from the alignment of orthologous DNA regions have been shown to be reliable guides for the identification of regulatory regions in many species. However, due to lack of sequence data these comparative analyses have not yet been performed with plants. As a preliminary evaluation of the usefulness of comparing cauliflower (*Brassica oleracea*) and *Arabidopsis thaliana* non-coding sequences to identify regulatory important regions, *B. oleracea* shotgun sequences were screened for those that overlapped the promoter of orthologous genes in *A. thaliana*. Thirteen *B. oleracea* shotgun sequences were aligned with *A. thaliana*, and well-conserved segments ranging from about 30 to 100 bases were found in most of the comparisons (10 out of 13). Even though the comparison set is small, this study indicates that there is likely to be conservation of promoter regions between *A. thaliana* and *B. oleracea* and that sequence comparisons across these two species will prove useful for the identification of regulatory regions.

1-17 High throughput gene silencing in Arabidopsis

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A major challenge in the post-genome era of plant biology is to determine the functions of all the genes in the plant genome. A straightforward approach to this problem is to reduce or knockout expression of a gene with the hope of seeing a phenotype that is suggestive of its function. Insertional mutagenesis is a useful tool for this type of study but is limited by gene redundancy, lethal knockouts, non-tagged mutants and the inability to target the inserted element to a specific gene. The efficacy of gene silencing in plants using inverted repeat transgene constructs that encode a hairpin RNA (hpRNA) has been demonstrated and can overcome many of the deficiencies of insertional mutagenesis listed above. To allow gene silencing to be used in functional genomics applications in a straightforward manner we have developed a series of vectors using the Gateway recombination system to facilitate the easy and rapid production of hp RNA constructs. The latest advances in design of these vectors will be described. The sequence of the Arabidopsis genome has shown that large portions of the genome are duplicated and therefore likely to be functionally redundant. This means that conventional single gene knockout approaches are unlikely to reveal the function of these genes. As gene silencing is sequence dependent it can potentially be used to silence multiple members of a gene family. We will present the results of experiments to define rules for construct design to silence multiple members of a gene family.

1-18 Development of a high density oligonucleotide microarray for *Arabidopsis thaliana*

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High density DNA microarrays have revolutionized gene expression studies of some organisms in recent years. We have produced an *Arabidopsis thaliana* oligonucleotide microarray consisting of 14,880 unique oligos each representing a different gene, using a non-contact inkjet deposition system. The 60 mer probes were designed using an algorithm that maximizes sensitivity and accuracy, while minimizing the possibility of cross-hybridization, thereby conferring high sequence specificity to each feature on the microarray. Sensitivity and differential expression accuracy have been measured using control oligonucleotide features on the microarray. With these microarrays, we routinely detect mRNA concentrations of 0.03pM (around 0.5 copies per cell in 106 cells). Differential expression experiments performed across multiple microarrays showed very accurate and consistent results (SD of LogRatios<10% of absolute values). Furthermore, a high correlation has been established between differential expression data obtained with these microarrays and data from other previously validated gene expression profiling platforms. Experiments that demonstrate the utility of these oligonucleotide microarrays in the study of Arabidopsis gene expression on a genome-wide scale will be described.

1-19 Dominant and semi-dominant mutants from RIKEN GSC activation tagging line

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The Plant Function Exploration Team is making a large number of activation tagging lines using *Arabidopsis thaliana* for comprehensive analysis of plant gene functions. By using the activation tagging technology, genes near the T-DNA insertion sites can be overexpressed by enhancers in T-DNA. The resulting overexpressor is expected to show a dominant or semi-dominant phenotype at T1 generation. In this study, we observed phenotypes of mutants at T1 generation from 40,000 lines that were found during preparing these lines. The category of phenotypes that we observed were as follows; 1) shape, number and size of rosette leaves, 2) timing of bolting, 3) shape of cauline leaves, 4) color of cotyledons and rosette leaves, 5) flower, 6) height of plant, and 7) apical dominance. We will report the frequency of mutant appearance, stability of mutant phenotypes at T2 generation and progress of researches.

1-20 The sizes and genome structures of the centromeric regions of *Arabidopsis thaliana* chromosomes 4 and 5

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We have determined the genome structure of the centromeric region of *Arabidopsis thaliana* chromosomes 4 and 5 by sequence analysis of BAC clones obtained by genome walking, followed by construction of a physical map using DNA of a hypomethylated strain (*ddm1*). The sizes of centromeric regions determined by the reduced recombination and cytogenetical method of the chromosomes 4 and 5 are approximately 5.3 and 4.5 Mb, respectively, which can be further divided into flanking region to the upper arm, central domain, and flanking region to the lower arm. The sizes of central domains of chromosomes 4 and 5 which primarily comprise blocks of the 180-bp repeats and *Athila* retrotransposons are approximately 2.7 and 2.9 Mb, respectively. Therefore, the entire length of chromosomes 4 and 5 become approximately 25.3 Mb and 30.5 Mb, respectively. These values are approximately 3 Mb longer than these estimated previously. The two flanking regions consist of various types of transposons, LINE, *gypsy*, of which *Athila* is the most prominent, and *copia*-type retrotransposons formed the highly nested structures. Several genes that are expressed were found in the flanking regions, but none in the central domain. Based on the characterization, here we present the detailed structure of the centromere of *A. thaliana* chromosome 5, which may represent the general feature of plant centromeres.

1-21 Development and evaluation of an Arabidopsis whole genome Affymetrix chip

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As part of our genome reannotation project, we have used the TIGR December 2001 annotation to develop an Arabidopsis “whole genome” gene chip. Recent developments in GeneChip technology that incorporate reductions in both element size and the number of probe pairs per gene allowed us to place ~22,700 probe sets on the new array. To best represent the >26,000 annotated genes, precedence was given to genes for which either expression evidence or a credible database match exist. Among the remaining “hypothetical” genes, we nominated every singleton and at least one member of each paralogous family. We also chose to represent very closely related gene pairs by a single probe set, thus allowing us to incorporate an additional ~1,000 genes. Altogether, the new ATH1 array represents ~24,000 genes. As a biological “reality check”, we used the array to analyze IAA-induced changes in gene expression. In response to 0.1 uM IAA, 241 genes were up-regulated and 79 down-regulated by a factor of 2 or more after 1 hr. For a small subset of these genes, the expression changes were also examined by real time PCR and reasonable agreement was observed. Of the ~4,900 hypothetical genes on the array, ~2,400 were observed to be expressed. A comparison between this array and the original 8K array of the ~1,200 expression ratios (+IAA:control) that were deemed significant changes on the 22K array showed a good correlation (R^2 ~ 0.8), with the ATH1 array generally reporting ~20% lower expression ratios. Supported by NSF.

1-22 Cloning and sequencing of cDNAs for hypothetical genes from chromosome 2 of *Arabidopsis thaliana*

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Approximately one quarter of the genes in the fully sequenced and annotated Arabidopsis genome have structures that are predicted solely by computer algorithms with neither nucleic acid matches from Arabidopsis expressed sequences or protein homologs from other species and are referred to as “hypothetical”. To test their expression under various conditions, we used cDNA populations from cold-treated, heat-treated and pathogen-treated plants, callus, roots and young seedlings. Of the 169 hypothetical genes tested to date, 138 are expressed in one or more of the cDNA populations. We obtained full-length cDNA sequences from 40 genes by sequencing and assembling their 5´ and 3´ RACE products. Twenty-nine of the genes have at least one cDNA assembly that precisely supports the predicted intron-exon boundaries, adding only 5´ and 3´ UTR sequences, with the other assemblies arising from alternatively spliced or unspliced introns and/or multiple polyadenylation sites. The cDNA sequences from another 11 (~27.5%) genes display differences from their predicted gene structures. These differences include 1) the predicted start or stop codon falls into an intron region; 2) the experimental cDNAs display intron-exon boundaries that are different from the predictions, including different numbers of exons and introns and different splice sites. In total, nine genes display more than one polyadenylation site. Therefore, most of the hypothetical genes examined to date are expressed in Arabidopsis and their cDNA sequences not only provide valuable information for validating their predicted structures but also for understanding their expression and regulation. Supported by NSF

1-23 Promoter trapping with firefly luciferase reporter gene in Arabidopsis

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T-DNA-mediated gene tagging is used intensively to analyse gene functions in higher plants. Various gene trap vectors are employed to identify tagged genes driving characteristic expression of fused promoterless reporter genes. We have developed a strategy to tag Arabidopsis genes with a promoterless firefly luciferase luc gene in the pTluc promoter trap vector that facilitates the characterization of temporal and spatial gene expression patterns in living plants using a sensitive CCD camera. Screening of over 10.000 transgenic plants for expression of the luciferase reporter lead thus far to the identification of 500 in situ gene fusions that display constitutive or tissue-specific, or stress-controlled temporal and spatial expression patterns. Tagged genes were identified by sequencing of PCR-amplified genomic DNA fragments flanking the T-DNA insertions. The application of the luc promoter trap system is illustrated by further characterization of stress-controlled genes coding for metabolic enzymes, membrane transporters and transcription factors. This work has been supported by OTKA grant no. T-029430 and the EU 5th framework Program, project no. GVE QLRT-2000-01871.

1-24 Sequence analysis and cDNA cloning of Arabidopsis F-box-containing proteins

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F-box proteins are a component of one of the E3 ubiquitin ligase complexes termed SCF. They regulate diverse cellular processes including cell cycle transition, transcriptional regulation and signal transduction by recognizing specific target proteins required for ubiquitination. In the last International Arabidopsis meetings, Gagne et al. Reported that 564 Arabidopsis F-box protein genes. We also found 569 F-box proteins in the Arabidopsis genome by database analysis. We classified the F-box protein by phylogenic tree analysis. Domain search analysis using SMART and Pfam-A databases revealed that F-box proteins contained LRRs, WD40 repeat and Kelch repeats that are implicated in protein-protein interactions. This analysis also resulted in the finding of several unique functional domains such as Tub, HLH and RING finger. However, many of F-box proteins did not contain any known domains. Therefore, these proteins were used to search the Pfam-B database to find novel domains and three putative ones were found. These domain search analyses led us to classify the Arabidopsis F-box proteins based on their C-terminal structures. To carry out functional analysis of F-box proteins, 90 cDNAs of F-box proteins were collected by RT-PCR and searching a RAFL collection (Seki et al. 2002), and cloned into Entry vector of Gateway system. Using the cDNAs, expression analysis and making transgenic plants are now on going.

1-25 Generation of Arabidopsis protein chips for antibody characterization

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The recent completion of the Arabidopsis genome sequencing now provides a basis for the further systematic analysis of gene expression and function. Therefore complex proteomic approaches using high-throughput technologies are useful. To complement classical proteomics, protein array technology is emerging as a tool to profile and functionally characterize proteins [1,2]. In this study, we developed and applied different strategies for cloning of Arabidopsis genes. Using gene specific primers, we cloned more than 100 different open reading frames in full-length, into GatewayTM-compatible E. coli expression vectors. The gene expression was performed in small volume (2 ml) in 96 well microtitre plates. Recombinant His-tagged proteins were purified in high-throughput and robotically arrayed onto coated glass slides. Using anti-RGS-His antibody followed by a fluorescence-labeled secondary antibody, the recombinant proteins could be specifically detected on the chips with a low background. The protein chips were used so far for the characterization of the specifity and cross-reactivity of monoclonal antibodies directed against plant proteins. Studies are under way to test further applications of Arabidopsis protein arrays and chips. This project is part of the GABI-project: Large-scale automated plant proteomics and is supported by the German Ministry for Research [BMBF-Förderkennzeichen: 0312274].

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1-26 A high throughput analysis and construction of the database for Arabidopsis transposon-tagging lines

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We are constructing Arabidopsis transposon-tagging lines using Ac/Ds system (Smith D. et al., Plant J., 10, 721-732, 1996), as an approach for functional genomics in post-sequencing era. We have obtained more than ten thousands of transposon-mutated lines so far. We extract genome DNA from each independent lines, amplify the adjacent DNA fragments by an adaptor-ligation-PCR, and sequence the amplified fragments to determine the insertion sites of Ds element in the genome. Introducing adaptor-ligation method allowed us to develop a high throughput analysis with a semi-automated system. (We thank Dr Alonso J.M. and Dr Ecker J.R. for advises to us for this method.) We are also making the database including the information for estimated positions of Ds insertion, the closest predicted-coding-regions and the results of homology search.

1-27 Arabidopsis POTATO tagging: Revealing gain-of-function phenotypes by promoter trapping

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We have designed a new activation tagging system for systematic gain-of-function screens in Arabidopsis. This system is based on the GAL4 system that was first used in Drosophila. A cell (or tissue) specifc promoter is used to direct the expression of a GAL4-VP16 synthetic transactivator in the cells of interest, and GAL4 in turn directs transcripts of the GAL4-responsive (UAS) target genes in an identical pattern. To identify genes affecting root development, a RCH1 (root clavata homolog 1)::GAL4 driver line was generated to direct the expression of the GAL4-VP16 synthetic transactivator in the Arabidopsis root meristem region. A passenger vector, which contains a UAS promoter oriented to the T-DNA right border, was transferred directly into the driver line to generate a population of transgenic lines for the gain-of-function screens. The system´s key feature is that a promoter-trap casette with a GUS reporter gene is used in the same passenger vector. This strategy allows us perform large-scale gain of function screen at the level of primary transformants by looking for (attenuated) GUS expression patterns in Arabidopsis roots, therefore we name our system as PoTATo tagging---Promoter Trap based Activation Tagging. A collection of 5500 transgenic lines was generated and screened, which allowed the identification of about 250 GUS-positive lines with various expression patterns. We are now analyzing the T2 generation of these lines. More detailed information for this system and further results will be presented on our poster during the meeting.

1-28 Determination of the biological functions of the *NPH3/RPT2* family

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Developmental plasticity is a key feature for the evolutionary success of sessile organisms since responses to environmental cues have to be precise and flexible to maximize their fitness value. The assembly of multimolecular complexes on scaffold proteins increases the speed and selectivity for specific signaling pathways utilizing signaling molecules otherwise common to multiple pathways. The NPH3 and RPT2 proteins of Arabidopsis are members of a novel plant-specific superfamily hypothesized to represent scaffold proteins. As part of NSF Arabidopsis 2010 Project, we are determining the biological functions of all members of the NPH3/RPT2 gene family (32 members). We are generating mRNA and protein expression profiles as well as loss- and gain-of-function mutants for each member. We will use these tools to design targeted physiological and developmental analyses to determine biological functions.A high-throughput cDNA-AFLP-based approach is being developed to examine mRNA profiles under a large number of conditions. In silico analysis for a minimal number of primer pairs giving gene-specific transcript-derived fragments based on fragment size and selective nucleotides is underway. We have identified potential loss-of-function mutant lines for many members of the family, and are currently verifying these lines and generating near-isogenic stocks. Gain-of-function alleles are being generated transgenically by driving expression of family members from their native promoters fused to 4 transcriptional enhancer elements from the CaMV 35S promoter. We hypothesize that this approach will result in overexpression of the genes in the correct temporal and spatial fashion, allowing for clearer interpretation of phenotypes relative to loss-of-function alleles.

1-29 Forkhead-associated domains of tobacco NtFHA1 transcription activator and yeast Fhl1 Fork-head transcription factor are functionally conserved

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*NtFHA*1 encodes a novel protein containing the FHA (Forkhead-associated) domain and the acidic domain in *Nicotiana tabacum*. NtFHA1 functions as a transactivator and targeted to the nucleus. The FHA domain of NtFHA1 is significantly homologous in sequences to that of Fhl1 forkhead transcription factor of yeast. *FHL1* was identified as a suppressor of RNA polymerase III mutations, and the *fhl1* deletion mutant exhibited severe growth defects and impaired rRNA processing. Ectopic expression of the FHA domain of NtFHA1, but not its mutant form, resulted in severe growth retardation in yeast. Similarly, expression of Fhl1, its FHA domain, or the chimeric Fhl1 containing the NtFHA1-FHA domain also inhibited yeast growth. The chimeric Fhl1 fully complemented the *fhl1* null mutation, demonstrating that the two FHA domains are functionally interchangeable. Yeast cells overexpressing the NtFHA1-FHA domain contained lower amounts of mature rRNAs, indicating that the growth retardation phenotype is related with reduced rRNA accumulation. These results demonstrate that the FHA domains of NtFHA1 and Fhl1 are conserved in their structure and function, and that the FHA domain influence rRNA maturation and cell cycle progression in yeast. NtFHA1 function in plants may be analogous with that of Fhl1 in yeast.

1-30 Microarray analysis of chitin elicitation in *Arabidopsis thaliana*

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Chitin oligomers, released from fungal cell walls by endochitinase, induce defense and related cellular responses in many plants. However, little is known about chitin responses in the model plant Arabidopsis. We describe here a large scale characterization of gene expression patterns in Arabidopsis in response to chitin treatment using an Arabidopsis microarray consisting of 2,375 EST clones representing putative defense-related and regulatory genes. Transcript levels for 71 ESTs, representing 61 genes, were altered ≥3-fold in chitin-treated seedlings relative to control seedlings. A number of transcripts exhibited altered accumulation as early as 10 min after exposure to chitin, representing some of the earliest changes in gene expression observed in chitin-treated plants. Included among the 61 genes are those that have been reported to be elicited by various pathogen-related stimuli in other plants. Additional genes, including genes of unknown function, were also identified broadening our understanding of chitin-elicited responses. Among transcripts with enhanced accumulation, one cluster was enriched in genes with both the W-box promoter element and a novel regulatory element. In addition, a number of transcripts had decreased abundance, encoding several proteins involved in cell wall strengthening and wall deposition. The chalcone synthase promoter element was identified in the upstream regions of these genes, suggesting that pathogen signals may suppress expression of some genes. These data indicate that Arabidopsis will be an excellent model to elucidate mechanisms of chitin elicitation in plant defense.

1-31 Comprehensive analyses of gene families involved in cell-wall construction in Arabidopsis

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The plant cell wall is a dynamic architecture constructed and maintained by many kinds of proteins, most of which are encoded by multi-gene families. Although a large number of cell-wall-related gene families have been identified to exist in Arabidopsis, there is little or no research in which expression profile of all members of a family have been characterized comprehensively because of difficulties in distinguishing individual genes within each gene family. To overcome the difficulty, a gene-specific oligo DNA microarray consisting of 762 cell-wall-related genes was designed based on the Arabidopsis genomic sequences. Using this gene-specific DNA microarray for celll-wall genes, we investigated their expression patterns during developmental processes in major organs. We also analyzed effects of various phytohormones on their expression levels. These analyses have revealed that many genes exhibit their own expression profiles developmentally and respond to hormonal stimuli differentially, though there are several gene groups with similar gene-expression profiles. Selected genes of interest in terms of expression profiles were subjected to a quantitative real-time RT-PCR analysis to quantify their expression levels more precisely. A combination of the microarray procedure and the real-time RT-PCR procedure afford excellentinsight into the precisely coordinated regulatory system in the transcriptions of the cell-wall-related genes during both organ development and responses to hormonal stimuli. Based on these findings we discuss the evolutionary diversification and conservation of the expression pattern in the cell-wall-related gene families.

1-32 Towards the identification of gene function based on computational promoter analysis: application to defense genes in *Arabidopsis thaliana*.

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Several studies have used genomic or proteomic approaches, to decipher how gene expression is affected during defense against plant pathogens. The availability of the complete Arabidopsis genomic sequence offers an opportunity to extract additional information from such expression data based on promoter analysis. We have conducted such a promoter analysis to identify motifs possibly involved in the regulation of gene expression by salicylic acid and ethylene, two key molecules involved in plant defense. Based on published microarray results, we collected sets of co-expressed genes. We confirmed the annotation of these genes and extracted 1kb of sequence upstream of each gene. This promoter sequence was examined with various motif-detection algorithms including CONSENSUS, MEME and the Gibbs sampling strategy. As a result, we have found six motifs, which are significantly over-represented in the up-regulated genes compared with down-regulated genes or random sequences. These motifs could be regulatory elements. Indeed, two of them include sequences, which are experimentally validated transcription factor binding sites. Next, we searched for these motifs in the complete Arabidopsis genome. We are currently in the process of testing genes identified in this search for induction by SA. We are developing experimental controls to test the biological relevance of these motifs. Our future aim is to identify novel genes involved in disease resistance and therefore complement our knowledge of resistance mechanisms.

1-33 Spreading of gene silencing as a high throughput system for gene function analysis in Arabidopsis

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RNA silencing is a homology-dependent RNA degradation process that follows recognition of double-stranded RNA (dsRNA). The dsRNA molecule can be replicating RNA viruses or transcripts of genes carrying inverted repeats (IR). The initiator dsRNA is cleaved by an RNaseIII protein, Dicer, into small 21-25 long RNAs (siRNAs). These siRNAs associate to a second RNase protein complex, RISC, which degrade single-stranded RNA having homology to the siRNAs. RNA silencing of transgenes is associated with a phenomenon that we refer to as spreading of RNA targeting. As a result of such a phenomenon all parts of transgene transcripts are targets of silencing even if the dsRNA initiator sequence corresponds to only a fragment of the transgene. Spreading of RNA targeting depends on the activity of the RNA-dependent RNA-polymerase Sde1 (Vaistij et al., 2002). We are developing a system to study function of target genes based on spreading of RNA targeting. This system is composed of plants carrying two transgenes. The triggering-transgene is an IR that will provide dsRNA to initiate silencing. The spreading-transgene is a fusion of sequences of the IR and a target gene. As a result of spreading of RNA targeting, siRNAs corresponding to the target gene will be produced. Then, these siRNAs will silence the endogenous gene transcript. This technology will be specially useful as a high through put system. A cDNA library can be easily cloned into the spreading-transgene vector and then used to transform Arabidopsis to produce a library of plants potentially silenced for any Arabidopsis gene.
[Vaistij, F.E., Jones, A. L. and Baulcombe, D.C. (2002) The Plant Cell 14, 857-867]

1-34 Gene expression profiling of cinmethylin-treated Arabidopsis seedlings

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We have recently initiated a program to study plant growth inhibitors with potentially novel modes of action. At present, commercially available herbicides target a relatively limited number of pathways and enzymes, thus the need exists to expand the repertoire of tools available for weed management systems. Transcriptional profiling represents a potentially effective means for elucidating inhibitor MOAs. Recently, our center has obtained evidence that the monoterpene cinmethylin acts via inhibition of asparagine synthetase, which plays a central role in the regulation of nitrogen metabolism in plants. Using a cDNA microarray comprised of 9,400 elements derived from *Arabidopsis thaliana*, we have examined global expression expression pattern changes in 10-day-old Arabidopsis seedlings exposed to cinmethylin concentrations sufficient to cause an approximately 50% reduction in root growth. The present work expands upon previous physiological and biochemical studies [Romagni et al., Plant. Physiol (2000); 123:725-732].

1-35 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* (60 000 primary transformants) from the “Station de génétique” (INRA, Versailles). Plant material produced from these lines is being used for the molecular characterisation of the T-DNA insertion sites ("FST" for Flanking Sequence Tag). After extraction of genomic DNA, FSTs are PCR amplified and sequenced (Balzergue et al., 2001). The sequences are then processed and compared to databases. FSTs are localized on the Arabidopsis genome and a graphical output indicates the relative position of predicted genes as well as FSTs. All the data (FSTs, molecular data) are stored and managed through an Oracle-based database (FLAG-DB) (Samson et al., 2002). Querys are made through a web server using a blast interface allowing to position any nucleotide sequence on the genome and to identify any FSTs in the vicinity or within the target sequence. The database, which is regularly updated, is available at **http://genoplante-info.infobiogen.fr**. To date (Feb. 2002) about 17 000 FSTs have been processed and 9000 FSTs are already available in the public database. These data allow an in depth analysis of the sequence specificity of insertion sites (IS) and brings new insights on the integration process (Brunaud et al., Submitted).

1-36 AraCyc, a biochemical pathway database for *Arabidopsis thaliana*

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AraCyc is a database being developed at the Arabidopsis Information Resource (TAIR) (http://www.arabidopsis.org) for the annotation of biochemical pathways in *Arabidopsis thaliana*. It presently features over 160 pathways that include information on compounds, intermediates, co-factors, reactions, genes, proteins and their sub-cellular locations. The pathways can be viewed graphically and the tools allow ‘zooming in’ to reveal more details about the reactions down to the molecular structures of all compounds and co-factors. An overview diagram gives a bird’s-eye view of all pathways in the database, and an expression viewer allows expression data to be overlaid on this diagram. The database is based on the Pathway Tools software developed by Peter Karp’s group at SRI International. It was initially built using the PathoLogic module with the MetaCyc database, a collection of pathways from over 150 species, as a reference database. More than a dozen plant-specific pathways, including carotenoid, brassinosteroid, and gibberellin biosyntheses have been added from the literature. A list of more than forty plant pathways will be added in the coming months. AraCyc will be available under the tools section of TAIR (http://www.arabidopsis.org/tools/aracyc), and can be accessed by June, 2002, using a standard web-browser.

1-37 Expression analysis by gene trap mutagenesis in *Arabidopsis thaliana*

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In order to analyze gene function systematically by utilizing the genome sequence information, we have generated a large number of T-DNA insertion lines. A newly constructed binary vector was used for transformation according to the vacuum infiltration method. The vector carries a *uidA* [beta-glucuronidase (GUS)] reporter gene for monitoring the promoter activity of inserted genes, a transposable element *Ds* for generation of a knock-out, and the *cis* sequences required for *Agrobacterium*-mediated transformation. As of March 2002, a total of 50,000 transgenic lines have been produced, and approximately 8% of the 20,000 plants tested for GUS activity exhibited positive staining. The insertion points that provide the donor loci for *Ds* transposition were determined by sequencing the flanking regions. The *Ds* elements were transposed in combination with the *Ac* element, and the expression of a luciferase reporter gene inserted into the *Ds* element was observed in gene-trapped lines. Among the generated lines, we are focusing on ones showing GUS staining in roots, and further characterizations of these lines are being performed.

1-38 Comprehensive genomic analysis of the CBL-calcium sensor/CBL- interacting protein kinase network

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Many developmental processes as well as signaling events in response to biotic and abiotic stimuli are accompanied by changes in cellular calcium concentration. Plants possess several sensing systems to detect and specifically forward the information generated by these calcium transients. We have recently described the family of **c**alcineurin **B**-**l**ike (**CBL**) proteins from *Arabidopsis thaliana* as novel signaling components involved in such processes and identified a specific group of serine-threonine protein kinases (**CIPK**s, **C**BL-**i**nteracting **p**rotein **k**inases) as targets of these sensor proteins. Comparative CBL-CIPK interaction studies of different CBL/CIPK combinations revealed that these proteins form a complex network connecting extracellular signals to defined cellular responses. Here we will present a comprehensive genomic analysis of the CBL/CIPK signaling network from *Arabidopsis thaliana*. This signaling network comprises of 25 kinases as targets of 10 CBL sensor proteins. Whole genome sequence comparisons covering 100 kb around each *CBL* and *CIPK* gene uncovered the nature and number of duplication (and deletion) events resulting in the current complexity. We have chosen the highly similar *CBL1* and *CBL9* loci as examples of a recent duplication to address the question of potential functional overlaps. Results of our expression analysis, interaction studies and mutant characterization suggest, that despite the significant similarity within the coding region both proteins exhibit sufficient functional diversity to enable functional analysis by reverse genetic approaches. The implications for the overall genetic analysis of complex gene families will be discussed.

1-39 Gateway binary vectors (pGWBs) for efficient transformation of plants

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With a gateway cloning technology, application of a recombination reaction of lamda phage in vitro, it is possible to make plasmids very easily without restriction enzymes and ligase. The cDNA clone in basic vector can be transfered various expression vectors keeping the reading frame by only one recombination reaction. In this paper, I report construction of new binary vectors available for gateway cloning technology and some applications. I used the pBI-Hm (pBI101 containing hygromycin resistant marker) for base and incorporated gateway casette. With this new binary vector, it is possible to clone the gene very easily. Next, I made various tag-adding binary vectors. These included GFP, 6xHis, FLAG, 3xHA, 4xMyc, 10xMyc and GST on either N or C terminal. With these vectors, it is possible to make binary vectors for expression of tagged protein by only one reaction. We will also report the results of protein expression in plants using these gateway binary vectors.

1-40 Using genomic approach for isolation and study of new MADS box transcription factors from Arabidopsis

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The completion of the Arabidopsis genome sequence opened for us new possibilities of study of various aspects of plant development. The MADS box proteins are eukaryotic transcription factors (TF), which play an important role in many biological processes such as the transcriptional control of cell-type specific genes and pheromone response in yeast (MCM1), and coordination of the transcription of the proto-oncogene c-*fos* in human (SRF). In plants this type of transcription factors is well known to play a role in transition of an inflorescence into a floral meristem, and in development of a flower (AP1, CAL, FUL, SEP1-3, AG and others). Using the HMMER software package the strictly conserved MADS domain (the DNA binding part of the MADS box TF), was used to search for new MADS box genes in Arabidopsis. Based on predicted gene sequences a set of gene specific primers was designed for each putative MADS box gene and used in PCR reactions with different plant tissue cDNAs. This resulted in isolation of 98 cds of MADS box TF, including those previously characterized. The new MADS box genes are found to be expressed in different plant tissues. We took an initiative to closely characterize a clade of *Agl*23 gene. The preliminary results of this analysis as well as some theoretical studies of the new MADS box TF will be presented.

1-41 Molecular and genetic characterization of the *REM* gene family in Arabidopsis

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The *REM* gene family is a moderately-sized gene family that encodes proteins of unknown function. The name of this gene family derives from the reproductive meristem (*REM*)-specific expression patterns of the first members of the gene family to be characterized. There are 43 *REM* genes in the Arabidopsis genome. Although *REM* family members are present in other plants (e.g. rice and Brassica) there are no bacterial, fungal, or animal homologs of these genes. The *REM* genes are distantly related to several classes of known plant DNA binding proteins, including B3 class of proteins like VP1 from maize, ABI3 from Arabidopsis, the auxin response factors (ARFs), and the RAV proteins. Based on this similarity, we postulate that the *REM* genes encode DNA binding proteins. One unusual feature is that the *REM* genes are clustered in the genome; nine *REM* genes (*REM1-REM9*) are clustered on chromosome 4, five *REM* genes (*REM10-REM14*) are clustered on chromosome 2 and three *REM* genes (*REM40-REM42*) are clustered on chromosome 5. Our studies on the *REM* genes began with the characterization of an enhancer trap (line21) that exhibited strong expression in the shoot apical meristem. The T-DNA in enhancer trap line 21, is inserted between two *REM* genes in the chromosome 4 cluster (*REM3* and *REM4*). The focus of our study has been to characterize enhancer trap line 21, the *REM* genes in the chromosome 4 gene cluster, and genes for which we have putative loss-of-function alleles.

1-42 Map-based cloning in *Arabidopsis thaliana*

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To rapidly determine the chromosomal location of phenotypic mutants, we have developed a strategy that makes use of the recently published physical AFLP map of Arabidopsis (Plant Physiology 127: 1579-1589) and the publicly available InDels and SNPs from Cereon (http://www.arabidopsis.org/Cereon/index.html). The AFLP map contains 1267 Col/Ler markers that are positioned on the Arabidopsis sequence. Around 50% of these are also polymorphic between Col/C24, Col/Ws and Col/Cvi. As the AFLP technique detects (many) more than one marker per reaction, it is very effective in the first steps of our map-based cloning strategy. This involves a linkage analysis with eight AFLP primer combinations that cover the genome with 87 well-dispersed markers. Twenty-two F2-mutant individuals are used to register linkage (and non-linkage!) of the gene-of-interest to these 87 AFLP markers. Typically a 3000-5000 kb region can be identified within one week. Further delineation of the area of interest to 400-600 kb can be accomplished within the second week by analysing AFLP markers that lie within the identified region for 100-150 additional F2-mutant individuals. In order to close in on the gene-of-interest, an F2 population of around 2500 plants is screened with two flanking, co-dominant InDel markers (insertion/deletion polymorphisms). The two markers can be analysed simultaneously on 96-well polyacrylamide gels. All recombinants are selected and used for final delineation with InDels and SNPs.

1-43 Development of a Transcription Factor Chip (TFC1)

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Experimentally verified or computationally predicted genes enable us to perform genome-wide analysis of gene expression. A small chip with about 1200 gene specific tags (GSTs) was designed, the Transcription Factor Chip 1 (TFC1). Specific primers for each GST were designed to produce amplicons of between 150-500 nucleotides. The chip design incorporates spiking controls that are laid out in a convenient pattern on the chip, and allow for the quantification of the applied RNA. The computational analysis of the array data will involve cluster analysis and database searches to define groups of transcription factors that have related expression patterns and functions. We intend to incorporate analysis methods that will enable us to organize the genes in a (hypothetical) transcriptional network.

1-44 Interphase chromosome territories in *Arabidopsis thaliana* revealed by chromosome painting

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FISH with chromosome-specific contigs of BAC clones enabled for the first time chromosome painting in a euploid plant, *A. thaliana* (Lysak et al. 2001, Plant J. 28). We have visualized interphase chromosome territories (CTs) by two-colour FISH with BAC contigs, spanning the short and the long arm of Arabidopsis chromosome 4 (17.5 Mb), to 2C nuclei isolated from root and leaf tissues. Four major patterns of territories have been found for the homologues of chromosome 4: (i) association of both homologous arms (44%), (ii) association of only the short-arms (9%), (iii) association of only the long-arms (17%), and (iv) complete separation of the homologous territories (30%). These values are compared with the expected ones according to a model for random spatial distribution (RDM) of CTs assuming a ~spheric shape of short and long arm territories proportional to their DNA content. Experiments are on the way to include the other chromosomes of Arabidopsis, various cell cycle, developmental and endoploypoidy stages as well as FISH with individual BACs from different chromosomal positions. This should reveal whether the spatial arrangement of interphase CTs fits the RDM, elucidate parameters which possibly lead to deviation from random distribution and clarify whether association of homologous CT reflects somatic pairing of homologues as observed in Drosophila.

1-45 Activation tagging in Arabidopsis: Identification of novel pistil/silique mutants by an EN-I tranposon tagging approach

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Activation Tagging in Arabidopsis has shown to be a powerful approach to identify new gene functions and to overcome genome redundancy. However, the commonly used system is based on T-DNA integration, which may lead to chromosomal rearrangements and silencing of the nearby genes. A new strategy to search for gain-of-function mutants has been followed avoiding the drawback of gene silencing. It is based on the maize En-I transposon insertion system containing a quadruple CaMV35S enhancer, which is made stable by out-segregation of the En-transposase using greenhouse based positive and negative selection markers. This stable population was screened for interesting pistil/silique mutants from which 3 have been selected, showing aberrations in pistil/silique development. In all 3 mutants, single transposon insertions were detected and segregation analyses have shown that they are all dominant mutants. The overexpressed genes present in the vicinity of the transposon element were cloned and further characterized molecularly. Suprisingly, the 3 mutants have similar phenotypes as those described for known loss-of-function mutants. The relationship between the activated genes and the corresponding loss-of-function mutants is determined by double mutant analyses and expression studies. The results of these analyses will shed new light on the regulation of genes involved in pistil/silique development.
This work has been financially support by CAPES- Brazil.

1-46 GATEWAY™ vectors for Agrobacterium-mediated plant transformation

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Gateway (Invitrogen, Carlsbad, CA) conversion technology provides a fast and reliable alternative to clone sequences in large acceptor plasmids. The technology is based on site-specific recombination reactions mediated by phage *lambda*. A set of Gateway-compatible binary T-DNA destination vectors have been described for a wide range of applications [Karimi *et al*. (2002), *Trends Plant Sci*. 7:193-195]. The backbone of these Gateway-compatible vectors is plasmid pPZP200. All types of T-DNA destination vectors are available with three plant selectable marker genes, conferring either kanamycin, hygromycin or bialaphos resistance. The Gateway recombination site for introduction of the fragment of interest is placed at the right of the T-DNA. To construct all vectors in this collection the reading frame A of the Gateway vector conversion system was used. For overexpression of a DNA sequence, the Gateway site was placed in between the promoter and terminator of the cauliflower mosaic virus 35S transcript. For cosuppression of plant endogenes, two series of destination vectors have been designed to generate inverted repeats. The intron cloned between the inverted Gateway cassettes was selected from an Arabidopsis database. Series of vectors have been constructed also for promoter analysis. In these vectors, a frame fusion between the coding regions of an enhanced green-fluorescent protein (GFP) and *beta*-glucuronidase was cloned downstream of the Gateway cassette. For localisation of particular proteins, another series of T-DNA destination vectors has been designed allowing N-, C-, or N- and C-terminal fusion of the protein of interest to the GFP protein.

1-47 High resolution 2-dimensional gels of lumenal, peripheral and triton extracted thylakoid proteins from *Arabidopsis thaliana*: Protein identification and expression analysis

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The thylakoid membrane system contains about 70 very abundant proteins organized in four major complexes (PSI, PSII, cytochrome b6/f and ATP synthase), carrying out photosynthesis. Many other proteins are present in the thylakoid proteome, which are involved in biogenesis, oxidative stress defense, proteolysis and regulation of photosynthesis. In this study we have quantitatively separated the hydrophyllic thylakoid proteome in three subproteomes - the soluble thylakoid lumenal proteome, the peripheral thylakoid proteome and a tightly membrane anchored peripheral proteome. The three subproteomes were separated by high-resolution 2-dimensional gels (18 cm, pI 4-7 and 6-11) and proteins were identified by Matrix Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry (MALDI-TOF MS) and nano Electro Spray Tandem Mass Spectrometry (nano-ESI MS/MS). The relative expression levels were determined by quantitative image analysis of Sypro Ruby stained gels. We will describe the function of the three proteomes. These reference gels will be used for a comparative proteome analysis to study abiotic stress responses.

1-48 A genomic approach for the characterisation of MADS-box genes in Arabidopsis

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The recent completion of the Arabidopsis genome opens up the opportunity for studies of regulatory networks, using a complete set of genes from an entire gene family. We present an approach for the characterisation of all MADS-box genes present within the Arabidopsis genome. Identification of MADS-box sequences in the Arabidopsis genome are typically done by screening of the databases using conserved motifs of the MADS-box region. To be able to be sure that the genes we study are actually expressed and not represent pseudo-genes we are developing a RT-PCR based method for the cloning of cDNA´s from all MADS-box genes studied. Once all cDNA´s have been obtained we will examine the co-ordinate expression of all MADS-box genes in wild type and conditional mutants of Arabidopsis, using microarray based expression analysis and *in situ* hybridisation. This will, not only, enable us to predict function and activity of the MADS-box genes, which are not yet characterised, but also to study the co-ordinate expression of all MADS-box genes and thus allow us to make predictions of how these genes act in a combinatorial manner to regulate plant development.

1-49 Analysis of interaction networks of transcription factors in *Arabidopsis thaliana*

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The completion of the *Arabidopsis thaliana* genome sequence suggests, that more than 5% of the estimated 30.000 genes belong to the group of transcription factors. To analyse the function of this large amount of uncharacterised proteins, methods are required which allow to standardise large scale screening procedures. As protein-protein interactions play a decisive role in almost all aspects of the structural and functional organization of the cell, the Yeast 2 Hybrid System provides a powerful tool to gain valuable information on a proteomic scale. An improved yeast two-hybrid interaction mating protocol investigated in our lab allows the simplified and efficient screening of at least 5 million double transformed cells. With this approach we aim to characterise interaction networks of the large R2R3 MYB-group of transcription-factors in A. thaliana. Only a small number of the 125 members are investigated so far, most of them seem to be involved in plant specific processes. First results of the Yeast Two Hybrid Screenings with members of this family are presented.

1-50 The Arabidopsis SeedGenes Project

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The SeedGenes Project (www.seedgenes.org) focuses on essential genes that give a visible seed phenotype when disrupted by mutation. Arabidopsis appears to contain 500 to 750 such EMB genes required for seed development and another 200 genes involved in seed pigmentation. Our goal is to coordinate the collection, analysis, and presentation of information on these genes based on cloning of mutant alleles. Project objectives are to approach saturation for cloned EMB and seed pigment genes; standardize phenotypic characterization of the corresponding mutants; understand the functions of these genes in growth and development; determine through expression studies and comparative sequence analysis why these genes are essential; and integrate this information into a project database accessible through the web. The first release of this database (March 2002) contains information on 100 genes and their knockout phenotypes. Included are 60 genes identified at Syngenta and another 40 genes described in the literature. Additional mutants and genes will be added to the project database at scheduled intervals over the next several years. The goal is to present information on 500 mutants defective in 300 EMB genes and another 100 mutants defective in 75 seed pigment genes. This project was made possible through a large-scale insertional mutagenesis program initiated 5 years ago at Syngenta (North Carolina) in collaboration with the Meinke laboratory. Initial results are described in Genetics 159: 1751-1763 (2001). The Virginia Bioinformatics Institute is coordinating gene expression, database, and web site functions. Funding is provided by the NSF 2010 Program and the Noble Foundation.

1-51 Identification of unstable transcripts in Arabidopsis by cDNA microarray analysis: Rapid decay is associated with groups of touch and clock-controlled genes

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mRNA degradation provides a powerful means for controlling gene expression in plants and other systems. Rates of decay help define the steady state levels to which transcripts accumulate in the cytoplasm and determine the speed with which these levels change in response to signals. When fast responses are to be achieved, rapid decay of mRNAs is necessary. Accordingly, genes with unstable transcripts often encode for proteins that play important regulatory roles. Although detailed studies have been carried on individual genes with unstable transcripts, there is limited knowledge regarding their nature and associations from a genomic perspective, or the physiological significance of rapid mRNA turnover in intact organisms. To address these problems, we have applied cDNA microarray analysis to identify and characterize genes with unstable transcripts in *Arabidopsis thaliana* (*AtGUTs*). Our studies showed that at least 1% of the 11,521 clones represented on Arabidopsis Functional Genomics Consortium (AFGC) microarrays correspond to transcripts that are rapidly degraded, with estimated half-lives of less than 60 min. *AtGUTs* encode proteins that are predicted to participate in a broad range of cellular processes, with transcriptional functions being over-represented relative to the whole Arabidopsis genome annotation. Analysis of public microarray expression data for these genes argues that mRNA instability is of high significance during plant responses to mechanical stimulation and is associated with a group of genes controlled by the circadian clock. Interestingly, two clock-controlled *AtGUTs* showed diurnal regulation of mRNA stability. These genes also exhibited altered mRNA levels in the Arabidopsis *dst* mutants (Perez-Amador *et al.* 2001. Plant Cell 13, 2703) suggesting a link between diurnal changes in mRNA levels and DST-mediated mRNA degradation in Arabidopsis.

1-52 Integration of Arabidopsis microarray expression in TAIR

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The Arabidopsis Information Resource (TAIR) (www.arabidopsis.org) is working towards incorporating microarray expression data to the resource with the purpose of providing access to Arabidopsis global gene expression information collected from databases or individual user submissions. The expression data will be searchable using array element/gene, RNA source, expression level, experimental design, methods, and experimenter information. In addition, a browsable format of the experiments and downloadable formats of raw, normalized, and summary data will be made available. In designing the structure of the database, special attention has been devoted to capturing information about the experimental design and the source of the RNA used for hybridization, based on the Minimal Information About a Microarray Experiment (MIAME) recommendations. This will allow retrieval of expression information based on, for example, tissue type and age, treatments, normalization method, or microarray technology used. The results will be presented on html pages describing the experiment, the biological material, and the methods employed, and will be linked to other relevant data in TAIR, such as gene ontology, publications, sequences, and maps. In addition, controlled vocabularies for anatomical and developmental terms that have been developed at TAIR will be used to associate to the expression information. The first set of data that are being incorporated represents the bulk of experiments performed by the Arabidopsis Functional Genomics Consortium (AFGC). We encourage the rest of the Arabidopsis community to submit their data to TAIR so that the whole plant community can benefit from this resource. To that end, we are developing submission forms for researchers to upload microarray data obtained using either Affymetrix or cDNA spotted technologies. Search and browse functions are planned for release in summer of 2002 and downloadable and data submission functions are planned for release in fall/winter of 2002. Files that contain information on the array elements used in AFGC and Affymetrix arrays, including annotation and their corresponding locus assignment, are currently available at ftp://tairpub:tairpub@ftp.arabidopsis.org/home/tair/Microarrays/.

1-53 New tools and features in TAIR

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The Arabidopsis Information Resource (TAIR) (www.arabidopsis.org) provides an integrated view of genomic data for *Arabidopsis thaliana*, incorporating data from a battery of sources including the Arabidopsis user community, the literature, and the major genome centers. TAIR has recently added the data produced by the AGI sequencing and annotation efforts from TIGR and all the Arabidopsis entries from GenBank to the existing collection of genes, markers, polymorphisms and map data. Other recent additions include gene family information and protein data. Our emphasis is now on incorporating functional annotations of genes and gene products, genome-wide expression, and biochemical pathway data. We are also developing tools to extract information from the literature in a systematic way, and building structured sets of controlled vocabularies to describe biological concepts in collaboration with other database groups (Gene Ontology Consortium, Plant Ontology Consortium). A significant new feature this year is the integration of the ABRC database functions and ordering system. Among the tools developed at TAIR, the most notable is the Sequence Viewer, which displays gene annotation, clones, transcripts, markers, and polymorphisms on the Arabidopsis genome, and allows zooming in to the nucleotide level. Other tools recently released include a bulk download tool for searching and downloading Gene Ontology functional annotations, sequences, protein data, and AGI locus code histories, and AraCyc, a tool for visualizing biochemical pathways. In addition, we have made significant improvements on the Pattern Matching tool, where researchers can search for any motifs and patterns in nucleotide or protein sequences, download the results, as well as additional data sets such as sequences upstream or downstream of transcripts.

1-54 Comparative analysis of the genes related to the signal transduction between Rice and Arabidopsis

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We have obtained 28K unique set of full-length cDNA and 15.823 clones were sequenced from the rice full-length cDNA project and homology search was made against NCBI GenBank data. About 4,600 clones were functionally annotated and 500 were categorized into the signal transduction genes. We have compared the existences of known signal transduction genes in plant (Arabidopsis and yeast) and animal (mouse, nematode and fly). (1) Membrane receptor (2) ligand receptor (3) MAP kinase cascade (4) calmodulin (5) G-protein (7) Cell cycle regulator were well conserved in rice, Arabidopsis and animals. On the other hand, (1) cytokine signal transduction (2) apoptosis signal transduction (3) Development inducing signal (4) Cell-to-Cell cross talk signal (5) Cell structure regulation (6) Adaptor protein network system are well-conserved signal transduction genes in animal kingdom but not found in Arabidopsis and rice. Rice specific signal transduction genes were found in some signal transduction systems of developmental and stress control. The similarities of these rice genes are low with those of Arabidopsis. We have also searched well-characterized transcription factors. Well-conserved transcription factors in animal but not existed in Arabidopsis (JAK1, Sos, bcl-2, ced-9, HES1, E1A, Grb2, p53, p21, Smad, Rb, EGR) were not found in rice yet. Otherwise, (E2F, myb, myc, jun), the common transcription factors in Arabidopsis and animals were found in rice. Plant specific transcription factor (TUBBY-like, CPP-like, E2F/DP, AP2/EREBP, NAC, ARF, YABBY) were also found in rice and Arabidopsis. We have also observed some transcription factors that were specifically changed in rice.

1-55 A list of Arabidopsis genes with mutant phenotypes

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This poster provides a list of Arabidopsis genes with loss-of-function mutant phenotypes. Meeting participants are encouraged to consult the list and to provide additions and corrections on the forms provided. The purpose of this project is to update the classical map of Arabidopsis mutants by transferring information from a recombination-based genetic map to a sequence-based physical map. Information provided by TAIR was used as a starting point to assemble a list of mutants with visible phenotypes that are disrupted in known genes with chromosome-based (At) numbers assigned through the Arabidopsis Genome Initiative. Mutant genes were divided into two major groups: those with a seed phenotype, which are being studied in detail through our NSF 2010 (SeedGenes) Project (www.seedgenes.org) and those without a seed phenotype, which represent genes with essential functions at other stages of the life cycle. The long-term goal of this effort is to determine the number and diversity of Arabidopsis genes that give a visible phenotype when disrupted by a loss-of-function mutation.

1-56 Design and databasing for the Complete Arabidopsis Transcriptome MicroArray (CATMA) project

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One major limitation of EST-based microarrays is cross-hybridization between genes in families. The CATMA project is at generating a collection of *specific* Gene Sequence Tags (GSTs) for every Arabidopsis genes. The GSTs are 150bp to 500bp-long amplicons of individual genes, obtained from genomic DNA over transcribed regions selected for their uniqueness in the genome as described : 1) Arabidopsis nuclear genes have been identified, using EuGène, a software proven to be very efficient in gene modeling in Arabidopsis. We thereby identified more than 29000 genes. 2) GSTs were designed using a new software (SPADS). The most specific region within each gene are searched. These regions are used as input in Primer3 to design the two primers for each GST. These primers, then the amplicon itself are tested for specificity. If the identity with a paralogue is over 70%, the amplicon is removed and the next one in the 3-5 orientation is processed. Up to now, GSTs were designed for 68% of the genes. A database containing all the information on the CATMA GSTs and their associated genes with links to EuGène and AGI entries has been built is publicly available at http://www.catma.org from June the 21st.

1-57 Towards functional genomics of plant cell walls using Fourier Transform InfraRed microspectroscopy

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Plant cell walls are complex composites of polysaccharides, proteins and phenolic compounds. Very little is known about the processes involved in the biosynthesis, transport and assembly of cell wall polymers. A large number of mutants with potential cell wall defects is available, and mutants for many putative glycosyl transferases potentially involved in polysaccharide biosynthesis have been isolated. A major limiting factor is the absence of reliable techniques for the analysis of cell wall polysaccharides compatible with small samples. Fourier Transform InfraRed microspectrocopy has been successfully used in the past to distinguish mutants with altered cell walls from the wild type. A limitation of this technique is that it is difficult to obtain information on the nature of the cell wall changes in mutants. We will show that this problem can be indirectly solved by using a procedure that involves the hierarchical clustering of a large number of mutants based on the FTIR spectra. The procedure was validated by the observation that alleles for the same locus were clustered in most cases, even in the absence of other observable phenotypic changes. We show that the clustering with mutants with known cell wall defects or wild type plants treated with specific inhibitors, can provide information on the nature of the cell wall defects in novel mutants. We will provide results showing that in this way novel mutants with defects in cellulose or pectin synthesis could be identified, as well as mutants with alterations in the cortical cytoskeleton. The technique can be used to characterise not only cell wall mutants but also cell wall changes during plant growth and development.

1-58 Phylogenetic analysis of the GATA family of transcription factor in Arabidopsis

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GATA transcription factors are a subgroup of DNA binding proteins broadly distributed in all the eukaryotes that recognize the sequence HGATAR. The GATA factors DNA binding domain is a class IV Zn finger motif in the form CX(2)Cx(17-18)CX(2)C followed by a basic region. In plants GATA DNA motifs have been proposed to be involved in light-dependent and nitrate-dependent control of transcription. An analysis of the Arabidopsis genome revealed the presence of 26 putative genes that encode for GATA factors. A phylogenetic analysis of the deduced 26 full-length GATA proteins, as well as the study of the genes intron-exon structure suggest the existence of three well defined subfamilies of GATA encoding genes in Arabidopsis. Subfamily I is formed by two-exons genes where the 3´exon encodes the complete zinc finger motif and the carboxy-terminal basic region. This subfamily is also characterized by the presence of a glutamine residue in the seventh position of the Zn finger loop. Subfamily II is constituted by two-exons genes where the sequence encoding the Zn finger has been split into the two exons. This subfamily is also characterized by the presence of serine at position 17 of the Zn finger loop. Finally Subfamily III is formed by two closely related genes with a non-homogeneous intron-exon composition and is characterized by the presence of the zinc finger domain at the amino-terminal end of the protein.

1-59 Proteome analysis of *A. thaliana*

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The recent completion of the genomic sequencing of *Arabidopsis thaliana* has paved the way for a systematic characterisation of the proteome of the first higher plant. To achieve this aim our attempt is based on the combination of 2-dimensional gel electrophoresis (2-DE) for separation and visualisation of proteins in crude tissue extracts, and mass spectrometric techniques for identification and characterisation of the isolated proteins The aim of the current study was on one hand to increase the number of proteins detectable by 2-DE analysis of plant tissues by optimisation of the protein extraction-, separeation- and staining- procedures. On the other hand we optimised protocols for efficient high throughput protein identification by MALDI-TOF MS. The results presented here give an insight into parts of the proteome of *Arabidopsis thaliana* by comparing different 2-DE spot patterns of expressed proteins in various tissues followed by their identification.

1-60 Characterisation and risk assessment of transgenic Arabidopsis th. L. modified in the flavonoid biosynthesis

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The safety of genetically modified crop plants has been brought into focus both by the industry and by the consumer society. One of the key concerns is the possibility of unintended metabolic perturbations due to the genetic modifications, which can alter health-influencing compounds in the transgenic food crops. In the EU-funded project, GMOCARE (QLK-1999-00765), different methods and approaches, such as functional genomics, proteomics and metabolite profiling, are exploited and tested for the detection and characterisation of unintended effects as a result of genetic modification. As a model plant, *Arabidopsis thaliana*, modified in the flavonoid biosynthesis, is included in GMOCARE. Flavonoids are secondary plant metabolites, which are believed to have possible health effects (cancer prevention). And, in addition to analyse for unintended effects in transgenic Arabidopsis, a better understanding towards the expression of genes involved in the flavonoid biosynthesis are studied as well. In this work, 2 transgenic Arabidopsis lines modified in the flavonoid biosynthesis have been developed and molecularly characterised. One with the antisense construct of *CHS* under control of the 35S promoter and one line with the antisense construct of *DFR* under control of an ethanol inducible promoter. The lines have been analysed for various transformation events and gene expression analysis using quantitative RT-PCR. Furthermore, gene expression analysis using the cDNA microarray technology has been performed. Finally, 2D PAGE analysis and HPLC analysis of flavonoids, with the aim of getting respectively, protein and metabolite profiles of the Arabidopsis GM-lines are in progress by other partners in the GMOCARE project.

1-61 Aramemnon: a novel Arabidopsis membrane protein database

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We are constructing a novel *Arabidopsis thaliana* membrane protein database that greatly simplifies the identification of membrane proteins and gene families, and the interpretation of membrane topology and subcellular localization predictions. The Aramemnon database contains all appr. 6000 *A. thaliana* membrane proteins/genes and the 679 membrane proteins from S*ynechocystis sp* The Aramemnon query options include:

- TIGR or NCBI accession numbers, free text, protein or DNA sequences (BLAST),
- membrane topology (including range of TM spans) and orientation of the N-terminus (non-/cytoplasmic),
- subcellular localization (mitochondria, plastids, secretory pathway). The Aramemnon output features include:
- protein, cDNA, and genomic DNA sequences from AGI/TIGR,
- display of transmembrane (TM) span predictions by seven different programs in a directly comparable, standardized graphical format,
- subcellular localization predictions by seven programs,
- a list of all Arabidopsis membrane proteins similar to a query sequence (>=30% sequence identity),
- a register of all similar *Synechocystis sp*. membrane proteins (>=30% sequence identity),
- the display of other membrane proteins located on the same BAC clone,
- a cluster analysis that presents a preview on the phylogenetic relationships among similar proteins based on pairwise distances,
- PubMed links to publications about the respective protein/gene.

The Aramemnon DB will be accessible on the internet later this summer. Future updates will include a sequence alignment tool, the comparative membrane topology graphical display of related proteins, improved annotation (gene names from publications, classification, functions) and a growing number of references. At a later stage also membrane gene/protein sets from other organisms will be incorporated.

1-62 Identification of candidate genes with a potential role in zinc tolerance and hyperaccumulation in *Arabidopsis halleri* by expression profiling using microarray chip technology

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Arabidopsis halleri has been described to occur in ‘Galmei´ floras on highly metal-contaminated soils in Central and Western Europe. In this so-called zinc hyperaccumulator plant, the accumulation of zinc in above-ground tissues has been reported to exceed 150 µmol g-1 dry biomass, which is about two orders of magnitude higher than the zinc concentrations found in common non-accumulator plants. In a hydroponic experiment, *A. halleri* was found to tolerate 300 µM zinc whereas biomass production of *A. thaliana* was significantly reduced after 4 d of exposure to 100 µM Zn in a hydroponic rooting medium. After exposure to 300 µM Zn for 4 d, leaf zinc concentrations were 220 µmol g-1 DW in *A. halleri* and 80 µmol g-1 DW in *A. thaliana*, as measured by inductively-coupled plasma atomic emission spectroscopy (ICP-AES). Expression profiling was performed to compare both *A. halleri* and *A. thaliana* with respect to changes in steady state transcript levels in response to exposure to high concentrations of zinc in the rooting medium. This was done by hybridisation to oligonucleotide microarray chips which contained probes corresponding to about 8000 Arabidopsis genes and expressed sequence tags (AFFYMETRIX GeneChipsTM). A number of differentially expressed genes were identified with predicted functions in metal homeostasis and signalling. Results will be summarized and discussed with respect to the applicability of this microarray system to species closely related to *A. thaliana*.

1-63 Comparative analysis of the transcriptional changes in the tobacco Cf-9-mediated defence response and the Arabidopsis FLS2-mediated defence response

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The tomato *Cf-9* gene confers resistance to races of the fungal pathogen *Cladosporium fulvum* expressing the *Avr9* gene. cDNA-AFLP display was previously used to identify transcripts whose expression patterns are altered during the Avr9- and Cf-9-mediated defense response in tobacco cell cultures. 290 cDNA-AFLP fragments out of 30,000 fragments inspected showed altered abundance in response to Avr9 (273 were inductions). These *ACRE* (*Avr9/Cf-9* rapidly elicited) genes are induced within 30 minutes of elicitation. The response of Arabidopsis cell cultures to the bacterial elicitor flagellin, and the synthetic peptide flg22, closely resembles the *Avr9/Cf-9* response in tobacco; reactive oxygen species are produced, MAP.kinases are activated, medium alkalinization occurs, PR-genes and signaling related genes are induced. In addition, structural similarities between FLS-2 and Cf-9 proteins suggest a common activation of defense pathways in response to flg22 and Avr9 product respectively. To compare and contrast the transcriptional changes in both experimental models, we have undertaken a large-scale analysis of gene expression in Arabidopsis cell culture (*Landsberg Erecta* ecotype) treated with flg22 peptide. Using Affymetrix microarray, we found that 204 probe sets representing 197 distinct genes were identified as being significantly regulated by flg22 peptide over a one-hour time course. These genes were called *FLARE* genes for FLAgellin Rapidly Elicited genes. To compare the transcriptional changes in both experimental models, we have investigated the behavior of Arabidopsis *ACRE* orthologs in response to flg22 elicitor. We will present the expression profiles of those genes and discuss their regulation through a clustering analysis approach.

1-64 Identification of novel members of the Arabidopsis cyclophilin gene family

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Cyclophilins are peptidyl-prolyl *cis-trans* isomerases, which were initially identified as the cellular receptors for the immunosuppressant cyclosporin. They are a class of highly conserved, ubiquitous proteins that have been suggested to play key roles in a number of cellular processes including protein assembly and signal transduction. To date, eight cyclophilin (or ROC, rotamase cyclophilin) genes have been identified in Arabidopsis; the abundance of cyclophilin isoforms suggests that they may carry out a range of diverse functions in addition to acting as protein foldases. To explore the possibility that additional Arabidopsis genes encoding cyclophilin-containing domains may exist, searches using the cyclophilin consensus sequence were carried out in both The Arabidopsis Information Resource and National Centre for Biotechnology Information databases. Sixteen novel cyclophilin-like genes were identified, bringing the total to twenty-four. EST sequences representing 22 out of the 24 genes have been deposited on databases, indicating that the genomic sequences are not pseudogenes. Members of the cyclophilin family vary greatly in size and complexity and are present in all subcellular compartments. They can be classified into distinct groups: the low molecular weight cyclophilins range in size from 18 to 28 kDa and include isoforms with predicted targeting to the endoplasmic reticulum, mitochondria and chloroplast. High molecular weight cyclophilins range in size from 40 to 96 kDa and contain additional domains such as WD40 repeats, tetratricopeptide domains, and arginine/serine rich regions. The abundance and functional divergence of Arabidopsis cyclophilin isoforms suggests that they may play a number of important roles in plant biology.

1-65 Characterization of the DST-mediated decay pathway in *Arabidopsis thaliana* utilizing genetic techniques in conjunction with microarray technology

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The *dst* mutants were originally isolated as specifically elevating the steady-state level and increasing the half-life of DST-containing transcripts. As such, they 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 abundance for two transgenes and an endogenous gene, all containing DST elements, when analyzed by RNA gel blot; however they show no visible aberrant phenotype. We have used DNA microarrays to identify genes with altered expression levels in *dst1* compared to the parental plants. RNA gel blot analysis confirmed the microarray data for all genes tested and was also used to catalog the first molecular differences in gene expression between the *dst1* and *dst2* mutants. These differences revealed previously unknown molecular phenotypes for the *dst* mutants that will be helpful in future analyses. Clustering analysis of genes altered in *dst1* exposed new co-expression patterns that prompt new hypotheses about the nature of the *dst1* mutation. Additional microarray experiments with *dst2* and *dst3* should provide further insight into the possible role of the DST-mediated mRNA decay pathway in plants. We have also initiated map-based cloning of the *dst* mutants. Cloning of the *DST* genes and characterization of their products and regulation, should present a powerful entry point into the machinery that recognizes and degrades DST-containing transcripts and the molecular mechanisms that govern rapid degradation of specific mRNAs.

1-66 The WRKY transcription factor superfamily in *Arabidopsis thaliana*

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WRKY transcription factors are plant specific zinc finger type DNA binding proteins. To date we have identified 75 *WRKY* genes in *Arabidopsis thaliana* of which at least 71 are expressed. Current data indicate that WRKY factors regulate gene expression by binding to *cis*-regulatory elements designated as W boxes (C/T TGAC T/C). W box elements have been shown to confer inducibility to certain elicitor- or wound-responsive promoters (e.g. parsley PR1, parsley WRKY1). Interestingly, W boxes appear to be "over-represented" in the promoters of pathogen/stress-induced Arabidopsis genes. Furthermore, genetic approaches demonstrate the involvement of WRKY factors in several biological processes such as pathogen/stress responses, senescence, and trichome development. We are interested in unraveling the diverse functions of this large family of plant specific transcription factors. This requires a group effort and the employment of various innovative and complementary approaches as will be presented.
http://www.mpiz-koeln.mpg.de/~somssich/wrky_webpage/Proteomics_Homepage_Startpage.html.

1-67 Transcriptome analysis of Arabidopsis innate immunity

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Bacterial flagellin has been recently identified as a pathogen-associated molecular pattern (PAMP) that is recognized by the innate immune system in such diverse organisms as insects, mammals and plants. In Arabidopsis, perception of flg22, a 22-amino-acid peptide, corresponding to the most conserved domain of eubacterial flagellin, requires the LRR-type receptor-like kinase FLS2 and activates a MAPK cascade signalling pathway. Flg22 treatment of seedlings induces production of reactive oxygen species, ethylene production, callose deposition, PR-genes expression and later growth inhibition. In order to identify flg22 early-responsive genes and to study their regulation, 2 week- old Arabidopsis seedlings (Col-0 ecotype) were treated with flg22 in the presence and absence of cycloheximide, an inhibitor of translation. Changes in mRNA expression levels were monitored using Affymetrix GeneChip Arabidopsis Genome arrays. Main classes of flg22 early-responsive genes encode transcription factors (such as WRKYs and EREBPs), receptor-like kinases and defense-related proteins. Interestingly, cycloheximide itself is sufficient to activate many of flg22 early-responsive genes. This hints at the possibility that the innate immunity response in plants also involves negative regulators with a rapid turnover. The identification and the analysis of flg22 early-responsive genes combined with molecular genetic and physiological studies will allow further dissection of molecular events in flg22 signal transduction pathway and address the impact of flagellin perception during an infection process.

1-68 GABI Primary Database: Database for plant genome data

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GabiPD (Primary database) is part of the German plant genome project GABI (Genomanalyse im biologischen System Pflanze), funded by the German Ministry for Education and Research (BMBF). The main objectives of GabiPD are to collect, integrate and visualize all relevant biological primary information from different GABI projects and to make data accessible via internet. Currently GabiPD contains a lot of different data, like sequence and SNP data, mapping information, results of microarray studies, and proteome analysis as well as information of T-DNA insertion lines. GabiPD, accessible via **http://gabi.rzpd.de,** allows fast access to the integrated data and the developed interfaces, e.g. GreenCards, enable simple database searches and clear visualization of search results. There is an initiative within GabiPD to link plant data of different GABI projects, especially Arabidopsis projects, as well as data from public repositories to reach a synergistic effect. A suitable interface called GenomeMatrix was established to display linkage between different Arabidopsis datasets. Particular emphasis within GabiPD is put on storing data of high troughput experiments, like gene expression data analysis. To ensure that integrated gene expression data are international comparable, the developed gene expression database schema is compliant with the schema proposed by the international Microarray Gene Expression Database group (MGED). The presentation will give you an impression of GabiPD contents, developed interfaces for data access and visualization as well as underlying database structures.

1-69 Proteinaceous inhibitors of cell wall/vacuolar ß-fructosidases and pectin methylesterases: Dissecting their multiple regulatory functions during plant development and stress responses

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The post-translational regulation of vacuolar and cell wall enzymes via inhibitory proteins has emmerged as a specific mechanism for rapid silencing of enzyme activity. Shortly after cloning of the first invertase inhibitor cDNAs from tobacco (Greiner et al., 1998, 1999) and the in vitro and in vivo proof of function of a cell wall and a vacuolar isoform (Nt-CIF, Nt-VIF), the direct sequencing of a Kiwi fruit pectin methylesterase inhibitor (IPME; Carmadella et al., 2000) revealed a significant sequence similarity to the invertase inhibitors. From this observation, three fundamental questions arise: 1) Are all or only some invertases and pectin methylesterases under control of inhibitory proteins; 2) During which developmental processes (or stress responses) are these regulatory mechanisms required; 3) What are the structural features relevant for their specificity towards CWI, VI or PME? In our experimental approach we use the sequenced A. thaliana genome as the platform to unravel the function(s) of 14 genes with significant homology to Nt-CIF, Nt-VIF or Kiwi IPME. In a functional genomics approach, we will characterize the targets for all 14 members of this diverse protein family and elucidate their roles during critical stages of plant development and in response to biotic and abiotic stress. Furthermore, by studying the structure and function of selected C/VIF and IPME members in vitro, we will gain knowledge on a yet poorly understood but important mechanism for post-translational control of enzymes involved in plant metabolism and growth control. First results of an ongoing investigation will be presented.

1-70 Regulated expression of the *ipt* gene in transgenic *Arabidopsis thaliana* to study cytokinin action

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We have used the LhG4 transactivation system to express the *ipt* gene of *Agrobacterium tumefaciens* in *Arabidopsis thaliana*. We demonstrate that the *ipt* gene is not expressed in the parental *Arabidopsis* reporter lines, but following transactivation after cross between reporter and activator lines the *ipt* gene is actively transcribed resulting in increased zeatin-type cytokinins, causing visible phenotypic alterations typical for increased cytokinin levels. Early seedling development appeared normal in *ipt* expressing progeny and root growth inhibition was not observed before 4 days after germination. Later *ipt* over-expressing plants developed a bushy phenotype with abnormal shoots and highly serrated leaves. To study changes in gene expression systematically we adopted cDNA macroarrays with around 12700 *A. thaliana* EST clones arrayed on nylon membranes. Filters were hybridized with probes derived from transactivated plants. As controls we used probes made from activator, reporter and wild type plants. We will present macroarray data describing these lines and discuss their relevance.

1-71 A useful source for rare transcription factor transcripts

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With the objective of isolating and annotating (new) *Arabidopsis thaliana* transcription factor cDNAs, as well as to expand the spectrum of accessible expressed genes, we searched for a suitable mRNA source for rare transcripts. Earlier studies suggested that the antibiotic protein synthesis inhibitor cycloheximide (CHX) induces transcript accumulation by increasing the stability of mRNAs. Here we report that cultured *A. thaliana* cells (At7) treated with CHX are a good source when searching for enhanced levels of transcription factor transcripts. The cells are growing heterotrophically in the dark and do not contain chloroplasts. We constructed a plasmid cDNA library starting from CHX-treated At7 cells and generated about 3000 ESTs. As expected, we observed the complete absense of photosynthesis-related transcripts. When compared to other existing sources of A. thaliana ESTs, a significant increase of transcription factor-related transcripts of about 100% was detected. These results were supported by macroarray experiments employing a specialised boutique filter array which represents about 1100 transcription factor genes. When hybridised with cDNA probes derived from CHX-treated and untreated At7 cells, a clear increase in numerous TF signals was observed.

1-72 Gene expression during ovary senescence and early fruit development in Arabidopsis revealed by DNA microarray analysis

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Ovary development ends in a programmed senescence process that is activated several days after anthesis, depending on the species. In contrast, application of plant hormones to young developing ovaries avoids the senescence and induces the transformation of the ovary in a seedless parthenocarpic fruit. Arabidopsis ovaries fully respond to gibberellic acid only during the first 3 days after anthesis, while senescence begins 7-8 days after anthesis. Functional genomic approaches such as DNA microarrays allows us to 1) characterize global changes in gene expression; 2) to identify marker genes, and 3) to assign new gene functions. We have used DNA microarrays containing more than 11,000 Arabidopsis cDNA clones, corresponding to 7,000-8,000 genes, to characterize changes in gene expression associated to ovary development and senescence and fruit-set. More than 500 Arabidopsis genes whose expression, at the mRNA level, is altered during ovary senescence and/or fruit development have been identified. We have selected a subset of genes based on their gene expression patterns and sequence information. Expression of these genes has been confirmed by Northern blot analysis. To determine the role of these genes during ovary development and senescence, fruit set, and early fruit development, alteration of gene expression by over-expression, T-DNA insertion, or RNAi will be carried out and the effects will be characterized.

1-73 Establishment of high-efficiency SNP-based mapping tools

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Single nucleotide polymorphisms (SNPs) are identified among the six *Arabidopsis thaliana* varieties Col-0, Ler, Cvi, C24, Nd, and Ei-2. These SNPs are used to establish highly efficient mapping tools. The specific goal of this project is the establishment of a framework marker set of approximately 100 evenly spaced SNP-based markers for use in rapid mapping of new mutations and for genotyping e.g. of near isogenic lines (NILs) and recombinant inbred lines (RILs), which are being developed in parallel. These tools will support QTL mapping and positional gene cloning. To this end, sites with average spacing of ca. 1.3 Mbp are selected from the Arabidopsis genome and subjected to comparative sequencing throughout the different varieties. Complete framework marker sets consisting of 112 evenly spaced polymorphic sites have hitherto been assembled for the variety combinations Col-0/C24, Col-0/Ler, and Col-0/Cvi (for Ler/Cvi in progress). The information gained in this way is used to set up efficient SNP detection procedures following a dual strategy: (1) Setup of arrangements for custom MS-based SNP analysis by service providers for the performance of large scale SNP analyses (e.g. for genotyping of entire RIL or NIL populations). (2) Implementation of efficient SNP assays based on multiplexed primer extension assays (SNaPshot) for small to moderate scale SNP analyses (e.g. for (re-) evaluation of individual lines as in the frame of specific NIL creation or for mapping of individual mutations). Upon validation, the data and the mapping tools (detection procedures) will be made publicly available.

1-74 Identification and characterization of genes that control petal development and senescence in rose and Arabidopsis plants

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Although the role of floral homeotic genes in the determination of petal identity is relatively well understood, very little is known about downstream events during petal development and senescence. Our research projects are aimed at improving our understanding of the molecular basis of petal development and senescence. We use a combination of 2 model plants: the rose plant that we incorporated into our research program as an ideal ornamental and applied model species and *Arabidopsis thaliana*, as genetic tool. We have performed a small-scale, targeted genomics program aimed at transcriptom survey of rose petals at different developmental stages (flower buds to senescent). Single-pass sequences were obtained from the 5 -ends of a total of 1794 rose petal cDNA clones. Cluster analysis identified 242 groups of sequences and 635 singletons indicating that the database represents a total of 877 genes. Putative functions could be assigned to 1151 of the transcripts. Expression analysis indicated that transcripts of several of the genes identified accumulated specifically at different petals developmental stages. We are currently using Arabidopsis as model plant for molecular genetic analysis of selected genes. The rose petal cDNA library and expressed sequence tag database represent a valuable resource for future research aimed at improving economically important rose characteristics such as flower development, longevity and scent production.

1-75 Comprehensive genome wide distribution and frequency of defined promoter elements

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Cis-regulatory elements are the sequences in a given promoter region bound by transcription factors affecting specific gene expression. A link between annotated genes and the occurrence of *cis*-regulatory elements in their promoters has correlated expressional data with the frequency of known *cis*-elements. Unfortunately, information about the frequency and distribution of *cis*-regulatory elements in the promoters of all annotated genes and within all chromosomes is not yet available. We developed different algorithms to analyze the occurrence of such elements in any given sequence and large datasets. Using these procedures resulted in a comprehensive annotation of the position and frequency of known *cis*-regulatory elements in the promoters of all annotated Arabidopsis genes and within the entire chromosomes. Pathogen inducible promoters and their *cis*>-regulatory elements are amongst the best studied promoter regions in plants. We can show that tested elements differ significantly in their frequency and distribution along the chromosomes and in predicted promoter regions. Whereas certain *cis*-elements occur nearly exclusively as monomers, other type of elements are more likely to form clusters. So far, our global analysis approach does reveal previously identified target genes. For example, the receptor-like kinase *FRK*/*SIRK* on chromosome II as well as a cluster of receptor-like kinases on chromosome IV were detected as possible target genes by their positive correlation of the frequency of *cis*-elements [W-boxes] in their putative promoter sequences. Thus, novel target genes should be found with this strategy.

1-76 Sequencing of T-DNA flankings for "in silico" detection of KO alleles (GABI-Kat-Kölner Arabidopsis T-DNA lines)

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To build up resources for efficient progress in plant genomics, a set of *Arabidopsis thaliana* T-DNA mutagenised lines with insertions sites identified by DNA sequencing is being generated. Columbia-0, the sequenced accession, was used as recipient for transformation. The resulting FSTs (flanking sequence tags) are mapped to the *A. thaliana* genome using BLAST, and the corresponding locus (gene) annotation is deduced. The results are integrated into a FST database describing which genes have been disrupted. Users can search the database for lines containing the KO allele which suites their needs. Finally, T2 seeds of single lines are delivered after in-house confirmation of the insertion locus. The goal of the project is to analyse 70.000 lines. We used a special Ti-based binary vector employing the sulfadiazine resistance marker to allow selection of transformed plants in the green house (Reiss et al., PNAS 93: 3094 1996). All steps from selection of resistant T1 plants to sequencing of PCR fragments representing insertion sites were optimised and integrated into an analysis pipeline. The throughput is about 700 lines per week, with greenhouse space as the limiting factor. A test experiment on several hundred lines indicated that the left border amplifications were more efficient and produced considerably more FSTs compared to those obtained with the right border. Segregation analysis using the resistance marker showed that 66% of the lines contain an insertion at only one genetic locus. The confirmation rate of insertions in the T2 generation is presently 78% of all initially detected gene hits.

1-77 The use of the split-ubiquitin system to detect plant protein-protein interaction

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Protein interactions are necessary for all biological processes. The split-ubiquitin system (USPS) allows the detection of interaction between soluble as well as membrane proteins and has successfully been employed to monitor interaction of yeast proteins. Here, we demonstrate the possibility of detecting plant protein-protein interactions using USPS. Analysis of protein interaction by USPS is based on cleavage of a reporter upon interaction of fusion proteins linked to N- and C- terminal halves of ubiquitin (Nub- and Cub-fragments, respectively). Reporter cleavage results in uracil-prototrophic and FOA-resistant yeast phenotypes. We tested the interaction between isoforms of the plasma membrane associated ARAC5, a small GTPase from Arabidopsis, and the cytosolic GAP1, a corresponding GTPase activating protein. Test for protein interaction on ura- and FOA+ selective media revealed, that GAP1 binds ARAC5 WT and the constitutively active, GTP-bound form of ARAC5, but not the constitutively inactive, GDP-bound form of ARAC5. These results correlate with data obtained in previous studies with other methods and demonstrate the potential of USPS for the analyses of plant protein-protein interactions.

1-78 Development and distribution of stocks at ABRC relevant to functional genomics

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The Arabidopsis Biological Resource Center (ABRC) maintains many stocks relevant to genome exploration and numbers of stocks are/will be received from 2010 Project grant recipients. Two major genomic resources are currently being received; the SALK sequence indexed T-DNA lines are currently arriving and will number 140,000 by late 2003. From these, it is expected that there will be indentified at least one knockout insertion line for a majority of the 25,000+ genes of Arabidopsis. Full-length Open Reading Frame clones are being received from the SSP Consortium (the R. Davis /J. Ecker/A. Theologis laboratories). 8,000 of these clones will be received in the next year. In addition, we have available T-DNA lines, associated DNA pools, the BAC genomic clones utilized for the sequencing projects and the EST collection representing ca. 10,000 Arabidopsis genes. 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 200,000+ of the above lines exist in quantities large enough for community-wide distribution as seeds for forward genetic screening. 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. Cooperation with the Arabidopsis Knockout Facility (AKF), U. of Wisconsin has also been in progress for some time. ABRC distributes the followup seeds associated with their PCR. ABRC is supported by the National Science Foundation.

1-79 Stock acquisition and distribution at the Arabidopsis Biological Resource Center, 2002

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The Arabidopsis Biological Resource Center (ABRC) cooperates with the Nottingham Arabidopsis Stock Centre (NASC) to collect, preserve and distribute seed and DNA stocks of Arabidopsis. ABRC stock information resides in the TAIR database(found at http://arabidopsis.org) with informatics support from the National Center for Genomic Resources (NCGR). Various seed stocks have been added to our collections in the past year, including: A) In excess of 60,000 SALK sequence-indexed T-DNA insertion lines. B) New mutant and transgenic lines, C) new natural accessions, D) TILLING lines and E) flank-tagged transposon lines. DNA stocks added to the collection include: A) Open Reading Frame (ORF) clones from the SSP Consortium, B) New clone accessions, C) clones for GFP expression and C) New libraries. During the past year, ABRC distributed 50,000 seed and 20,000 DNA stocks to researchers. ABRC is supported by the National Science Foundation.

1-80 DOF transcription factors: On the way to identify their downstream genes

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Transcription factors (TFs) regulate the expression of downstream genes and thereby contribute to the establishment of complex traits in higher plants, including developmental features, cell differentiation, biosynthetic pathways and adaptation to environmental stresses. The plant specific DOF TF family has 37 members sharing a highly conserved cysteine rich motif for a single zinc finger. Several members of this family have been analyzed in maize, barley, tobacco and potato, but only few DOFs such as OBP1-3 and DAG1 were investigated in Arabidopsis yet. The aim of this project is to identify the downstream genes of selected DOF transcription factors in Arabidopsis and to integrate them with the interesting visible phenotypes including altered leaf and flower morphology. Based on protein similarities the DOF family was subdivided into eight subfamilies. Arabidopsis lines were created overexpressing 29 different DOFs representing these subfamilies. Considering their visible phenotype and their position on a distance matrix tree, different lines were selected representing five DOFs that were investigated on Arabidopsis GeneChips. Interestingly the data clearly show a distinct subset of downstream genes in each transgenic situation. However corresponding to their relative distance on the matrix tree, some of the DOF TFs share common downstream genes suggesting partially overlapping functions.

1-81 The PPR family: A huge novel family of proteins involved in organellar gene expression

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The sequencing of the Arabidopsis genome has given a huge boost to the identification of novel organellar proteins. Prediction programs such as TargetP and Predotar allow the whole genome to be searched for putative targeting sequences. Rapid methods for testing these candidates by GFP or RFP fusion have been developed. Using these approaches, we have identified a huge family of plant proteins containing characteristic tandem arrays of a 35 amino acid motif which we have termed the pentatricopeptide repeat (PPR). There are more than 450 members of the PPR family in Arabidopsis making it the largest protein family in the genome of which the basic role remains a mystery. The majority of these proteins are predicted to be targeted to chloroplasts or mitochondria. A few PPR proteins are known from other organisms, and the evidence so far suggests that they interact with specific mRNAs, playing a role in mRNA processing or translation. The members of this family make excellent candidates as specificity factors for various processes involving organellar mRNAs, notably RNA editing and nuclear restoration of CMS. We are employing the latest functional genomics techniques (high-throughput recombinational cloning, RNAi, microarrays, etc.) to try and ascertain the role of these proteins in plants. Experiments underway include subcellular localisation of proteins by GFP and RFP fusions, analysis of T-DNA insertion mutants, bacterial expression of PPR proteins for biochemical and structural studies, and a search for macromolecular partners via purification of tagged complexes from transgenic plants and two- and three-hybrid screens in yeast.

1-82 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 community with the sequence of about 26,000 genes. For about two-thirds of these genes, some idea of the function of the gene 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, 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 use 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. We expect to clone about 5,000 genes during the 2-year project. Two classes of ORFs will be cloned: - 5,000 ORFs will be amplified from full-length cDNA clones sequenced during a Génoscope/INRA/Invitrogen collaboration. - 75 ORFs will be amplified directly from first strand cDNA during a pilot project. The methods have been optimised in the laboratory on the PPR gene family. The PPR family is a large family of organelle-targeted proteins characterized by the presence of tandem arrays of a 35-amino-acid repeat. The function of these proteins is unknown although they are presumed to play various roles in organelle gene expression (see Small et al. poster).

1-83 Herbicide target discovery: A model for functional genomics

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The goal of this project is to examine the cellular basis of plant growth and development by saturating for insertional mutants defective in essential genes. Over 123,000 T-DNA lines generated at Syngenta and 10,000 Ds transposon lines from Cold Spring Harbor Laboratory have been screened for embryonic and seedling lethal phenotypes. We have used TAIL PCR to recover flanking sequence from a large collection of lethal mutants. Approximately one-third of the recovered sequences have homology to genes with known functions, another third have homology to genes encoding hypothetical proteins, and the remainder have no significant homology to sequences in GenBank. Essential genes identified to date fall into all but two of the 15 major functional categories outlined by Bevan et al. [Nature (1998) 391:485]. As expected many of the identified genes have basic cellular functions. Based on sequence similarities, several identified genes fall into the disease/defense category, consistent with an important role for such genes in growth and development.

1-84 Chromosomal microarrays for functional analysis of the Arabidopsis genome

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The completed sequence of the Arabidopsis genome offers the opportunity to assess, on an unprecedented scale, the impact of genome structure on gene activity and chromosome function in a higher eukaryote. We are particularly interested in studying the effects of chromatin modification on transposon activity and transposon-mediated regulation of genes, which could account for a variety of epigenetic phenomena. To this end, we are constructing a genomic tiling microarray for Arabidopsis chromosome 4, by printing on glass slides sequential 1kb fragments from the 17Mb of known sequence. In parallel, we have developed robust techniques to profile transcriptional activity, DNA methylation and histone modifications. Based on a pilot microarray covering 1.5 Mb in and around the heterochromatic knob present on the short arm of chromosome 4, we have shown that the putative chromatin remodeling mutant..*ddm1*.. leads to a dramatic increase of transcriptional activity that is mainly restricted to sequences located in the knob and/or to related repeats found elsewhere. These correspond mainly to transposons, hypothetical genes and non-annotated regions, in keeping with the gene-poor composition of heterochromatin. Moreover, we have shown that histone H3 methylation patterns in Arabidopsis heterochromatin depend on the SWI/SNF-like gene DDM1, as does DNA methylation. These results provide the first evidence of an interconnection between DNA methylation, chromatin modification and chromatin remodeling and suggest that although often considered genetically inert, heterochromatin may provide a considerable reservoir of hidden transcriptional activity.

1-85 Analysis of the gene expression patterns in Arabidopsis leaf under cold stress using SAGE method

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The genes expressed within an organism determine its physical characteristics. A variety of internal or external factors can modulate these gene expression patterns, leading altered physiologic or disease states. Serial Analysis of Gene Expression (SAGE) method was applied to characterize the global patterns of gene expression in the normal Arabidopsis leaf. A total of 21,280 SAGE tags representing 12,049 unique tags, among which only 3,367 genes (27.9%) matched the Arabidopsis cDNA or EST sequences in the DNA database, were sequence identified. In the highly expressed class, as expected, many tags matched with well-characterized photosynthesis related genes. Functional analysis of annotated tags indicated that a significant proportion of the genes with predicted functions were involved in energy and metabolism. To systematically analyze differential transcription profiles under cold stress, the gene expression patterns from cold-treated leaves were analyzed using a similar scale. A comparison of the tags present in the cold-treated leaf with those identified in the normal leaf revealed 70 transcript tags that were overexpressed, by P value < 0.01, in the normal leaf cells and 162 tags that were similarly overexpressed in the cold-treated leaf cells. The genes up-regulated by cold treatment were mainly multiple COR genes, lipid transfer genes, alcohol dehydrogenase and novel genes, whereas down-regulated genes were mostly photosynthesis related gene. In general, many genes involved in metabolism, protein synthesis, transport facilitation and cell rescue/defence/cell death/aging are induced and many genes involved in energy and metabolism. The expression levels of several cold responsive transcripts were confirmed by Northern analysis. These results provide extensive insight into the molecular and cellular background of cold–response gene expression and information valuable for the characterization of novel genes involved in the freezing tolerance of a plant.

1-86 Arabinogalactan proteins: the search for function

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AGPs are high-molecular-weight proteoglycans that contain 1-10% protein [1] and belong to a large family of wall (glyco) proteins, the Proline/Hydroxyproline (Pro/Hyp)-rich glycoproteins (P/HRGP) [2]. In Arabidopsis, there are at least 47 genes encoding AGP protein backbones and most of these are predicted to be GPI-anchored [1,3]. They can be divided into sub-classes based on their predicted amino acid composition [3]. We have identified 13 classical AGPs, 10 AG-peptides (predicted mature protein backbones of 10-17 amino acids), 3 basic AGPs (that include a short Lys-rich region), and 21 FLAs (putative cell adhesion molecules) [1]. AGPs are implicated in diverse roles in plant growth and development but no precise function has been assigned to any AGP. Our aim is to identify functional roles for these glycoproteins. We have DNA insertion mutant lines from various populations and RNAi and ectopic expression lines for many AGP genes, however, no phenotype has been observed in any of these single modified gene lines. Our research strategy now is to create multiple mutants and to carry out the most appropriate molecular, biochemical and genetic experiments, based on expression pattern data, on the mutant and transgenic plants in order to identify functions.

1. Gaspar Y et al., 2001, Plant Mol Biol 47, 161
2. Bacic A et al., 2000, In “Cell and Devel Biol of AGPs". Kluwer Academic Press, p 11-23
3. Schultz CJ et al., 2002, Plant Physiol, accepted

1-87 Key genes throught Keygene Transcript Databases

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At Keygene N.V., so far, two transcript databases have been produced: an Arabidopsis transcript database and a tomato transcript database. The procedure for the production of transcript databases is:

1. Transcript profiling using cDNA-AFLP (R) on different tissues in different (stress) conditions;
2. Isolation of fragments;
3. Sequencing of fragments;
4. Quality control and homology searches.

In Arabidopsis, 65,000 expression profiles haven been produced and sequence analysis resulted in 40,000 good quality sequences. Coverage estimates vary between 62 and 66% of 26,000 Arabidopsis genes.
Transcript databases serve:

- as reference databases for projects that utilize cDNA-AFLP technology;
- to determine expression profiles of known genes;
- to determine expression profiles of individual family members;
- to select tissue specific or inducible promoters based on expression profiles.

Use of transcript databases will be illustrated.
(R) (cDNA)-AFLP is a registered trademark of Keygene N.V., Wageningen, the Netherlands.

1-88 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, 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. After users design primers to their specific gene, 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 3500 first round and 2600 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

1-89 A high-throughput reverse genetics system for Arabidopsis

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To increase the speed of functional genomics in Arabidopsis a high-throughput modified TAIL-PCR protocol was developed and used to amplify DNA fragments flanking the T-DNA left borders in approximately 100,000 transformed lines. These TAIL-PCR products were sequenced and compared to the Arabidopsis genome to generate a table that associates the positions of T-DNAs in each line with promoters and coding regions of Arabidopsis genes. Analysis of this table suggests that the collection contains insertions in the coding sequences of at least 12,000 genes, and insertions in upstream promoter sequences of at least 17,000 genes. Predicted T-DNA insertions identified using modified TAIL-PCR and sequencing show a bias toward insertions in upstream promoter sequences, and outside of coding sequences. Candidate T-DNA insertion mutants in this collection can be identified using gene name searches of the table, or by BLAST comparisons of the sequence of any gene of interest against the flanking sequence database. Insertions are confirmed by simple PCR assays of individual lines. Of 347 lines tested to contain T-DNAs in candidate genes, the predicted insertions were confirmed in 257 (74%). This resource has been named GARLIC (Gilroy Arabidopsis Reverse Library Insertion Collection) and is available to the academic community for research purposes.

1-90 Transcriptome analysis of high-light-treated wild-type and photoprotection mutant plants

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Plants require light to drive photosynthesis. The absorption of excess light however, can be harmful, leading to photoinhibition and photodamage. Plants have therefore evolved photoprotection mechanisms to cope with conditions of high light (HL). One photoprotection mechanism is thermal dissipation of excess absorbed light, measured as non-photochemical quenching of chlorophyll fluorescence (NPQ). A large part of NPQ is dependent on the thylakoid pH gradient, de-epoxidized xanthophylls and Photosystem II subunit S protein. Studying NPQ- and xanthophyll-deficient mutants showed that NPQ is important for immediate photoprotection but is not necessary for long term acclimation to HL. To identify and characterize other photoprotection mechanisms, we did a large-scale expression analysis using the Affymetrix GeneChip® arrays on wild-type and NPQ- and xanthophyll-deficient mutant plants, treated with short (4 hours) and long (10 days) periods of HL. Of the 8734 sequences represented on the array, 15% changed expression by 2-fold or more after exposure to HL compared with LL, in all genotypes. The majority of change was seen after 4 hours of HL. Apparent return to LL expression levels occurred after 10 days, indicating HL acclimation. Activity of a number of gene classes will be discussed. A number of transcription factors and other potential regulatory genes showed a HL-affected expression pattern and were chosen for further study.

1-91 Arabidopsis encyclopedia using full-length cDNAs and its application for functional genomics

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Full-length cDNAs are essential for the correct annotation of genomic sequences and for the functional analysis of genes and their products. Using the biotinylated CAP trapper method, we constructed full-length cDNA libraries from Arabidopsis plants1),2) and isolated 155,144 RIKEN Arabidopsis full-length (RAFL) cDNA clones. The 3-end ESTs of 155,144 RAFLcDNAs were clustered into 14,668 nonredundant cDNA groups, about 60% of predicted genes3). We also obtained 5´-ESTs from 14,034 nonredundant cDNA groups and constructed a promoter database. We determined full-length cDNA sequences of 8,554 RAFL cDNA clones in collaboration with the Arabidopsis SSP group of the USA(PI: Drs. Ecker, Theologis and Davis) as of March 25, 2002. We are planning to determine full-length cDNA sequences of ca. 15,000 RAFL cDNA clones. After determination of full-length cDNA sequences, the RAFL cDNA clones are available from the RIKEN Bioresources Center. We have also used the full-length cDNAs for the microarray analysis of expression profiles of Arabidopsis genes under drought, cold and high-salinity-stresses4). 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. (2002) Science 296: 141-145
4) Seki et al. (2001) Plant Cell 13: 61-72

1-92 Proteomic isolation of stress-related proteins in Arabidopsis

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With the accumulation of vast amounts of DNA sequences in databases, it is now apparent that a factory approach is desirable to gain a comprehensive understanding of complex biological processes. In this sense, proteomics is a powerful technology of functional genomics for the large scale analysis of proteins. Our research efforts have been made to integrate proteomics into the systematic and comprehensive investigation of biological processes in plants. We aim to identify proteins in Arabidopsis that are up- or down-regulated in both protein and phosphorylation levels in response to abiotic stresses. We are interested in a nuclear proteome that includes DNA binding proteins such as transcription factors. The presentation will describe the methodological establishment for purification of the nuclear/DNA binding proteome and proteomic analysis of the isolated proteomes.

1-93 Biological resources of Arabidopsis distributed from RIKEN BioResource Center

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In 2001, BioResource Center (BRC) was established in RIKEN Tsukuba institute. The aims of BRC is the collection, preservation and distribution of biological resources in Japan to promote the research in life science. Plant biological resources are distributed from Experimental Plant Division (http://www.brc.riken.go.jp/lab/epd/index.html). Recently, we start the distribution of the mutant Arabidopsis seeds (1100 mutants from Ac/Ds transposon-tagging line) and Arabidopsis 7000 full-length cDNAs (Seki et al., Science 296: 141-145, 2002) that have been deposited from RIKEN Genomic Sciences Center. The T-DNA tagged Arabidospsis mutant seeds (activation tagged lines) will be ready for distribution in nearly future. We present the feature of these resources and the distribution system of RIKEN BRC.

1-94 Transcription activation systems for regulated transgene expression

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Using *Ac/Ds* transposons we have constructed an enhancer trap to generate patterns of expression of the synthetic transcription factor LhG4. Following integration near an enhancer in the genome, LhG4 expression is detected by GUS and GFP genes under control of the pOp promoter (the target of LhG4) at an unlinked site. Any gene can then be misexpressed in the same pattern as LhG4 by introducing it to Arabidopsis under control of the pOp promoter. We have also generated a T-DNA based enhancer trap population that incorporates an improved LhG4 cassette. These populations are giving rise to unique patterns of pOp-GUS expression 10 - 20% of transformants. We have also generated a dexamethasone-inducible derivative of LhG4 called LhGR and have improved the reporter cassettes for expression of genes of interest. The LhGR system appears to be physiologically silent in Arabidopsis and does not evoke the morphological abnormalities associated with other dexamethasone-inducible systems. We will present the characteristics of the LhG4 and LhGR systems and a summary of the LhG4 expression patterns that are currently available.

1-95 Nitric oxide mediates iron induction of ferritin accumulation in *Arabidopsis thaliana*

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Nitric oxide (NO) is a signaling molecule that plays a critical role in the activation of innate immune and inflammatory responses in animals. During the last few years, NO has also been detected in several plant species and the increasing number of reports on its function in plants have implicated NO as an important effector of growth, development, and defense. We investigated the possible role of NO as regulator of iron homeostasis in plant cells, in particular its involvement in the regulation of *A.thaliana* ferritin, an iron-storage protein which accumulates in response to iron increase. The infiltration of the NO-donor sodium nitroprusside (SNP) in Arabidopsis leaves induces accumulation of ferritin both at mRNA and protein level. Iron is not necessary for this NO-mediated ferritin transcript accumulation, since SNP is still able to induce the accumulation of ferritin transcript in Arabidopsis suspension cultures pretreated with the iron chelants DFO or ferrozine. However, NO is required for iron-induced ferritin accumulation, as the NO scavenger CPTIO prevents ferritin transcript accumulation in Arabidopsis suspension cultures treated with iron. The pathway is ser/thr phosphatase-dependent, necessitates protein synthesis and is cGMP independent. Most important, NO mediates ferritin regulation through the IDRS sequence of the *Atfer1* promoter responsible for transcriptional repression under low iron supply. NO, by acting downstream of iron in the induction of ferritin transcript accumulation is therefore a key signaling molecule for regulation of iron homeostasis in plants.

2-01 *FPF1* regulates flowering time by GA signalling

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The transition to flowering is one of the most visible phase changes during plant development and has been studying in great detail during the last years. We have identified a gene encoding a small protein of 104 amino acids (FLOWERING PROMOTING FACTOR 1) that plays an important role for the onset of flowering.
FPF1 is expressed during the transition to flowering in the peripheral zone of apical meristems and in floral meristems. In order to identify the function of *FPF1* we have searched for knockout mutants and have obtained En1 tagged mutants that flower later than the corresponding wild type plants. Constitutive expression of *FPF1* leads to early flowering and the transgenic plants phenocopy to a great extent either plants which are treated with GAs, or mutants that show a constitutive GA signal response or an enhanced responsiveness to GAs, indicating that *FPF1* might play a role in the activation of a GA pathway. Further evidence for a role of *FPF1* in GA signalling comes from molecular studies of genes that are regulated by GAs, which showed that the expression of *FPF1* has an influence on the regulation of these genes. In addition, we can show that the biosynthesis of GAs are directly influenced by *FPF1* expression. Results from crosses of *FPF1* over-expressors and *fpf1* mutants with ga mutants will be discussed.

2-02 Light-mediated regulation of CONSTANS protein

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The CONSTANS (CO) protein promotes the transition to flowering in response to long day photoperiods. *CO* encodes a transcription factor with two B-box type zinc-binding motifs at the amino-terminal end and a carboxyl-terminal domain that is conserved in related genes but is of unknown function. We have previously shown that the expression of CO is regulated by both circadian clock and photoperiod so that its overexpression promotes early flowering in short and long days. The flowering-time genes FT and SOC1 are activated by CO and this effect is antagonised by the floral repressor FLC. The peak of expression of FT in 35S::CO plants in a 24 h cycle is placed to the illuminated part of the day when compared to wild-type plants and is minimal during the night. On the other hand, FT expression in 35S::CO is absent in dark adapted plants and reaches maximum levels upon illumination, demonstrating a light-dependant activation of CO. We have also demonstrated that CO is an unstable nuclear-localized protein and that overexpression of CO in blue light is toxic to plants. This toxicity is related to its activity because it is not observed when mutant forms of CO are overexpressed. To further characterise light mediated regulation of CO at both the transcriptional and postranscriptional levels, mutations in all major photoreceptors have been introduced into 35S::CO plants and the activity and stability of the protein in different light conditions have been analysed.

2-03 Flowering-time gene *FD* encodes a bZIP protein which is required for the function of a floral pathway integrator *FT*

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Flowering in Arabidopsis is regulated by several interacting pathways which are integrated by genes such as *FT, SOC1/AGL20*, and *LFY* to finally execute the floral transition. FT protein has similarity with mammalian protein known as phosphatidylethanolamine binding protein or Raf-1 kinase inhibitor protein, however, its biochemical function remains to be elucidated. We have been interested in factors acting downstream of *FT*. The late-flowering gene *FD* is a good candidate of genes acting downstream of *FT*. The *FD* activity seems to be required for the precocious-flowering phenotype of *35S::FT*, because otherwise very weak *fd-1* mutation strongly suppresses *35S::FT* phenotype. Suppression effect is specific to *FT*, because *fd-1* does not strongly affect early-flowering phenotype caused by overexpression of *LFY* (Nilsson et al., 1998) or *SOC1/AGL20* (our results). We identified the *FD* gene by fine mapping of *fd-1* in *35S::FT* background. *FD* gene encodes a bZIP transcription factor of the ABF/AREB/GBF4 subclass. *fd-1* has a nonsense mutation which eliminate most of the C-terminal part including bZIP region, and is likely a null allele. About 1-Mb region containing *FD* on chromosome 4 has a duplicated region with a paralog of *FD* (*FDP*) on chromosome 2. This may partly explain very weak phenotype of *fd-1*. FD protein can interact with FT protein in yeast. FT is likely to play a key role in regulation of FD function by protein-protein interaction. Results of genetic and molecular characterization of *FD* will be presented.

2-04 Molecular mechanisms of FT and TFL1 signalling

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FT and TFL1 are members of a family of widely conserved proteins involved in signalling in mammals and plants. In Arabidopsis, FT promotes flowering, while TFL1 acts antagonistically and delays flowering. The TFL1/FT family of proteins includes RKIP (Raf Kinase Inhibitor Protein), which modulates signalling through several diverse kinase cascade pathways in mammals. In order to understand how FT and TFL1 signal in Arabidopsis, a combination of biochemical and yeast two-hybrid screens have been carried out. Efficient purification of both FT and TFL1 was achieved using a TEV protein A (TAP) affinity tag from whole cell extracts. Although sufficient amounts of the tagged protein could be visualised by 1D SDS PAGE, no interacting proteins were detected. This suggests that FT and TFL1 may interact only transiently with their signalling partners. Two hybrid analysis has confirmed the interaction of FT and TFL1 with numerous 14-3-3 proteins, which has been previously observed by our collaborator Eliezer Liefshitz for the tomato TFL1 homolog SP. An extensive two-hybrid screen with both FT and TFL1 has revealed several new interacting proteins, which will be discussed.

2-05 Floral transition mediated by a Chromatin Remodeling Protein: Lesson from the study of Arabidopsis early flowering mutants

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A collection of Arabidopsis early flowering mutants have been isolated from an activation-tagging T-DNA population in short day conditions. Studies on these mutants revealed that one group of the mutants show early flowering phenotypes regardless of photoperiodic conditions, while the other show certain-photoperiod-specific early flowering phenotypes. These early flowering mutants could also be grouped into three different types based on preliminary circadian analyses; group1 having shorter photoperiods, group2 having longer photoperiods, and group3 having wild-type-like photoperiods. Majority of the early flowering mutants mapped at loci where flowering genes have not yet been reported and genetic/molecular analyses revealed that both activation-tagged floral activators and loss-of-function floral repressors could be isolated from this type of screening. Introgression of *FRI* into these early flowering mutants allowed the identification of several suppressors of *FRI/FLC*-dependent floral regulatory pathway. Identification of the target gene for one (*ef57*) of these *FRI/FLC*-suppressors showed that *FLC* function is regulated by a SWI2/SNF2 family chromatin remodeling protein. *EF57* expression was detected in shoot apices but not in root apices. *EF57* was not regulated by *FRI/FLC*, but *FLC* was regulated by *EF57* suggesting that *EF57* is an upstream regulator of *FLC*. Given the fact that *FLC*-dependent floral transition is under the control of epigenetic regulations by environmental cues such as vernalization, regulation of *FLC* by *EF57* implies a possibility that *EF57* could be a central regulator in this floral transition pathway. Consistent with this possibility, *EF57* expression was differentially regulated in many early flowering mutant backgrounds.

2-06 Cytological events prior to lateral root initiation

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An essential feature of growing plants is the development of a root system, which consists of a primary root and a number of lateral roots (LRs) emerging from the primary root. LR formation passes through a spatially and temporally regulated division pattern, by which only a limited number of differentiated pericycle cells, opposite the protoxylem poles, are involved (1,2). The pattern of LR formation has been very well characterized in the model plant *Arabidopsis thaliana* (L.) Heynh. (3). Coordinated anticlinal asymmetric divisions in a few pericycle cells, followed by periclinal divisions will result in the formation of a LR primordium and eventually in a full grown LR. Since there is growing evidence that asymmetric divisions are essential in the determination of new cell fates, we are currently focusing on this particular step in lateral root formation. Using several approaches (in vivo GFP-analysis, nuclear DAPI staining, microscopic analysis of sections with LM and TEM) we try to elucidate the cytological characteristics of a cell preparing for re-entry in the cell cycle prior to an asymmetric division. A parallel project is focusing on the identification of genes that play an important regulatory role in this process. We will present data on several anatomical and cytological features of the pericycle, e.g. changes in cell shape of an "(in)activated" pericycle cell, nuclear movement and shape, DNA content, changes in cytoskeletal structure, cytoplasm shifts and changes in organelles.

(1) Beeckman *et al.* (2001). *Journal of Experimental Botany* 52: 403-411
(2) Casimiro *et al.* (2001). *The Plant Cell* 13: 843-852
(3) Malamy & Benfey (1997). *Development* 124: 33-44

2-07 Control of proliferation, endoreduplication and differentiation by the Arabidopsis E2Fa/DPa transcription factor

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In mammals, the decision of cells to continue or stop dividing depends largely on the activity of the E2F-DP heterodimeric transcription factor. Inhibition of E2F-DP activity arrests dividing cells at G1, whereas E2F overexpression forces serum-starved cells to enter S phase. Recently, E2F and DP genes were discovered in plants. Because of their described effects in mammals, we postulated that plant E2F and DP genes regulate the proliferative identity of plant cells. To prove this hypothesis transgenic lines were generated overexpression either the E2Fa or the DPa gene. DPa transgenic lines were phenotypic indistinguishable from control plants. In contrast, E2Fa transgenics displayed enlarged cotelydons due to an increase in cell number. This increase was at least partial due to prolonged cell division, correlated with a delay of cell differentiation. The phenotype was strongly enhanced in plants co-expressing DPa and E2Fa. In E2Fa-DPa transgenics many more cell divisions were observed. Simultaneously endoreduplication was enhanced, resulting in at least 2 extra endocycles. A model is presented explaining the different observed phenotypes triggered by E2Fa-DPa overexpression. Because E2Fa-DPa transgenic plants arrested early in development, we argue that controlled exit of the cell cycle is a prerequisite for normal plant development.

2-08 Control of germination and lipid mobilisation by COMATOSE, the Arabidopsis homologue of human ALDP

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Embryo dormancy in flowering plants is an important dispersal mechanism that promotes survival of the seed through time. The subsequent transition to germination is a critical control point regulating initiation of vegetative growth. We show that the Arabidopsis *COMATOSE* (*CTS*) locus is required for this transition, and acts, at least in part, by profoundly affecting the metabolism of stored lipids. CTS encodes a peroxisomal protein of the ABC transporter class with significant identity to the human X-linked adrenoleukodystrophy protein (ALDP). Like X-ALD patients *cts* mutant embryos and seedlings exhibit pleiotropic phenotypes associated with perturbation in fatty-acid-metabolism. CTS expression transiently increases shortly after imbibition during germination, but not in imbibed dormant seeds, and genetic analyses show that *CTS* is negatively regulated by loci that promote embryo dormancy through multiple independent pathways. Our results demonstrate that CTS regulates transport of acyl-CoAs into the peroxisome, and indicate that regulation of CTS function is a major control point for the switch between the opposing developmental programmes of dormancy and germination.

2-09 A regulatory role for salicylic acid in the transition to flowering in Arabidopsis

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The transition from vegetative to reproductive stage in plants depends on endogenous cues and environmental factors. Stress, either from abiotic or biotic origin, stimulates the transition to flowering. A well characterized stress-related molecule such as salicylic acid (SA) regulates defense responses as well as developmental processes. We proposed a new regulatory role for SA on flowering time in Arabdiosis. SA-deficient plants, known to be defective in pathogen-activated defenses, exhibit late-flowering phenotypes both under long- and short-day photoperiods. The late-flowering phenotype is stronger under short-days correlating to an elevated expression of *FLC* gene. Late-flowering is partially rescued by raising SA levels in SA-deficient plants not affected in SA accumulation (*eds5* and *sid2* mutants) but not in SA-deficient plants unable to accumulate it (transgenic nahG plants). Vernalization of SA-deficient short days-grown plants reduced *FLC* gene expression and flowering time both in *eds5* and *sid2* mutants and in nahG plants, likely through a SA-independent pathway. Under long-days, the late-flowering phenotype of SA-deficient plants correlates to a lower expression levels of photoperiod-dependent flowering time genes *CO*, *FT* and *SOC1* and higher expression of *FLC* than Columbia wild-type plants. A model is proposed where SA regulates the transition to flowering in Arabidopsis through both photoperiod-dependent and –independent pathways.

2-10 EMF repression of embryo and flower development in Arabidopsis

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The *EMBRYONIC FLOWER* (*EMF*) genes, EMF1 and EMF2, are required for maintaining vegetative development and repressing flower development. EMF1 encodes a putative transcriptional regulator and EMF2 a Polycomb Group (PcG) protein homolog. Using the GeneChip technology, we examined expression profile of emf mutants. High degree of overlap in expression changes among the emf1 and emf2 mutants confirms functional similarity between the two EMF genes. Comparison of differential expression profiles of emf mutants to developmental stage-specific expression profile revealed a striking similarity in the genetic program of emf mutants and the Arabidopsis flowers, indicating a commitment of germinating emf seedlings to the reproductive fate. Ectopic expression of flower organ identity genes and late embryo abundant protein genes in germinating emf seedlings revealed that in wild-type plants, vegetative development is maintained via a global EMF repression of the flower and the embryo development programs, directly or indirectly. Gene expression analysis showed no clear regulation between EMF1 and FLOWERING LOCUS T (FT), LEAFY (LFY), TERMINAL FLOWER 1 (TFL1), CONSTANS (CO), suggesting that EMF1 regulation of flower organ identity genes is not mediated by these genes. Temporal and spatial expression pattern of LFY:: Glucuronidase (GUS) and APETALA1 (AP1)::GUS confirms that AP1 ectopic expression in emf seedlings is independent of LFY. Based on these findings, a new mechanism of EMF-mediated floral repression is proposed.

2-11 Activation of *ECL1* promotes flowering and alters leaf morphology in Arabidopsis

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We have isolated *ecl1D* (*Early Curly Leaf* 1) by activation tagging screen, which shows early flowering under long days. The homozygous *ecl1D* plants flowers with average 6 leaves with a formation of terminal flower. In addition, the early flowering phenotype is observed independent of photoperiod. Time course experiments showed early flowering of *ecl1D* is partially resulted from the precocious upregulation of *FT* and *SOC1 (=AGL20*). Activation of *ECL1* also affects leaf morphology, showing curly leaf phenotype under long days; however, the formation of curly leaves is delayed under short days. The early flowering and altered leaf morphology of *ecl1D* are attenuated in hemizygous plants, suggesting *ECL1* acts semi-dominantly. Interestingly, strong curly leaf phenotype is observed in hemizygous *ecl1D* plants in *35S::FT* background, which implies *FT* enhances the curly leaf phenotype of *ecl1D*. *However*, *SOC1*, which acts parallel with *FT* downstream of *CO*, does not enhance the curly phenotype. Investigation of genetic interaction of *ECL1* controlling flowering time is in progress.

2-12 FCA, an RNA binding protein that controls the floral transition, autoregulates its expression by alternative polyadenylation

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The Arabidopsis nuclear RNA binding protein, FCA, promotes reproductive development. The FCA pre-mRNA is polyadenylated at two different positions. The use of the proximal poly(A) site in intron 3 produces a truncated transcript (beta), whereas polyadenylation at the distal site yields three different transcripts (alfa, gamma and delta). FCA protein expressed from the nature gene is undetectable in lines over-expressing FCA from an intronless transgene. The analysis of the native FCA transcripts shows that only transcript beta is detectable in these lines. In contrast, the level of beta is reduced in fca loss of function mutants. This indicates that FCA negatively autoregulates its expression by actively promoting use of the proximal poly(A) site in its own pre-mRNA. As a consequence, the production of gamma, the only transcript functional in flowering time control, is limited. We have previously found that the level of FCA expression is limiting for flowering time. Therefore, this regulation has a functional consequence for flowering time control. The presence of intron 3 in a translational FCA:GUS fusion restricts GUS activity to regions of active cell proliferation in a wild-type background. When introgressed into a fca-1 mutant background, however, GUS expression can be detected earlier and with a broader distribution. Therefore, FCA controls the temporal and spatial patterns of its own expression. FCA physically interacts with the polyadenylation factor FY through its WW domain. The negative feedback requires an intact FCA WW domain and a functional FY protein. When this negative regulation is removed, ectopic over-expression of FCA in Arabidopsis results in pleiotropic phenotypes and premature senescence, providing an additional rationale for negative regulation of FCA expression.

2-13 Role of *FRI* in repressing the floral transition

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The majority of the Arabidopsis accessions are classified as winter annual types, that is they flower late unless their flowering is accelerated by a long period of cold temperature in a process known as vernalization. Many genes control flowering time in Arabidopsis, however the vernalization requirement segregates as a single gene trait mapping to the FRIGIDA (FRI) locus. We have been studying two questions: 1) When and where is FRI needed in plant to delay flowering? Clonal analysis has been used to address this question. Sectors where FRI is specifically expressed on a background that does not carry an active FRI allele have been generated in the plant using the heat-shock inducible Cre-lox recombinase system. The analysis of plants where Cre was induced at the embryo stage shows that early FRI expression is sufficient to give the late-flowering phenotype. More detailed analyses are being done to investigate the requirement for FRI in space and time. 2) What is the molecular basis of the allelic variation at FRI? Early flowering accessions not carrying the two previously described deletion alleles have been chosen for further analysis. In the Cape Verde Island accession a single bp mutation results in an in-frame stop codon that provides evidence for a third independent evolution of early flowering through mutation at FRI. Analysis of FRI in the early flowering accessions Shakhdara, Kondara, Kz-9 and Wil did not reveal any polymorphisms likely to result in loss of function. Genetic analysis is being undertaken to investigate the role of FLC, a gene whose functionality is necessary for the activity of FRI, in the flowering time of these accessions.

2-14 Gene chip expression analysis of *bp*, the *KNAT1* loss of function mutant

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Plant development depends on the activities of apical meristems, groups of indeterminate cells that develop into different organs. Studies on *knotted1* and related homeobox (*knox*) genes have identified important roles for hese transcription factors in meristem initiation and maintenance. In particular we sought to determine the target genes of *KNAT1*, a *knox*-like gene, by examining the null mutant. The *KNAT1* loss of function mutant, *brevipedicellus* (*bp*), shows reduced stem elongation and irregular node distance. Moreover the top of the inflorescence is flat with an umbel shape and the siliques point downward. Using the Affymetrix Chip Gene technology, we compared the gene expression between *bp* mutant and Columbia wild type plants. Two week old seedlings were used as starting material for the Chip analysis. Of 8300 genes tested, 59 are differentially expressed between mutant and wild type. We identified genes coding for biosynthetic enzymes belonging to three major pathways and DNA binding proteins. We further found a substantial group of genes that have previously been identified to be hormone regulated. Taking these findings together with previous results that show effects of *knox* genes on hormones, it is likely that KNAT1 is integrated with both hormone signaling pathways and hormone homeostasis.

2-15 Activation-tagging and overexpression of *LSH1*, a member of a novel gene family, suggesting its role in photomorphogenesis and floral meristem determination

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We isolated a light dependent short hypocotyl mutant, lsh1-D from screening Arabidopsis activation-tagged lines. This mutant shows shorter hypocotyl than wild type in light but normal hypocotyl length in darkness. LSH1 gene is located close to the right border of the T-DNA insert linked with the mutant phenotypes and its expression in lsh1-D is indeed much enhanced. LSH1 encodes a protein of a novel gene family which has been found only in plant species and shows no significant homology with any proteins of known function. To further examine the role of LSH1 in plant development, we generated LSH1 sense and anti-sense transgenic Arabidopsis under CaMV 35S promoter. As lsh1-D, the sense LSH1 overexpressors also shows exaggerated light responses. More over, higher expression of LSH1 causes transformation of flowers into inflorescence shoots, a process known as floral meristem reversion, which suggests that LSH1 might function to suppress the natural developmental process of inflorescence meristem to floral meristem. LSH1 is localized in nucleus and LSH1 gene expression was predominantly detected in shoot apical meristem, floral meristem and floral organs.

2-16 Regulation of flowering time by COP/DET/FUS photomorphogenic repressors in *Arabidopsis*

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The transition to flowering is one of the most complicated processes in plant development, which requires the coordinated regulation of many flowering-time genes responding to appropriate day lengths. We examined the possible roles of COP/DET/FUS photomorphogenic repressors in the regulation of the transition since their mutants flower early in SD. Using the viable *cop1-4, det1-1, and fus9-2* mutants, we have analyzed the flowering time of double mutants that combine with late-flowering (*cry2-1, co-1, gi-1, ft-1, fca-9, ld-1 and soc1*) and early-flowering mutations (*ap1-10, tfl1-11, phyB-9 and spy-3*) in LD and SD. The genetic and molecular analyses of double mutants demonstrates that COP1/DET1/FUS9 attenuate the activation of *SOC1* by CO in photoperiod pathway and COP1/FUS9 weaken the repression of *FT* by FLC in autonomous pathway under LD. In SD, the *co* mutation is epistatic to the early-flowering effects of *cop1/det1//fus9* mutations while the other early- or late-flowering mutations are additive, possibly due to a fact that the daily cycle of *CO* expression begins 4-h earlier than the wild type. It indicates that the COP/DET/FUS are involved in the phase-switching mechanisms of both photoperiod and autonomous pathway in LD, and also the circadian rhythm of *CO* expression in SD.

2-17 Control of floral induction and plant yield: role of the *MSI4* gene coding for a WD-repeat protein

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Work on a dioecious plant, *Silene latifolia*, allowed the cloning and characterisation of the first gene localised on a plant sex chromosome: *SLY1*. The product of this gene is preferentially expressed in meristems and floral organ primordia and seems to be involved in the control of cellular proliferation. Five homologues of *SLY1-MSI1-5* , for *Multicopy Suppressor* of *IRA 1*, according to yeast nomenclature - are found in the *Arabidopsis* genome. All code for WD proteins homologous to the Retinoblastoma-binding protein of mammals, involved in the cell cycle control. One mutant for the *MSI4* gene, the most likely ortholog of *SlY1*, was found in the T-DNA tagged collection (INRA at Versailles, France, WS ecotype). The *msi4* mutant presents a late flowering phenotype and an increase in biomass and seed production. The phenotypic analysis shows that flowers are larger in the mutant than in the WT and exhibit an increased number of organs (mainly sepals and petals). Furthermore, cytometric analyses indicated that the level of ploidy is not significantly modified in the *msi4* mutant. *msi4* is allelic to fve, a late flowering mutant identified previously (José Martinez-Zapater, personal communication).
Ongoing experiments suggest that MSI4 proteins are potential regulators of flowering time via mechanisms that control cell proliferation (rate, window...). Genetic, molecular and cellular work will be presented in our attempt to define the function of the *MSI4* gene.

2-18 The characterization of a polygalacturonase gene (*PGAZAT*) specifically expressed during floral organ abscission in *Arabidopsis thaliana*

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Abscission is the shedding of organs from the body of a plant. In order for this process to occur it is necessary that specialised cells are formed and these have been shown to be anatomically distinct from the distal or proximal cells adjacent to them. During organ separation, an increase in the activity of several hydrolytic enzymes has been shown, one of these is the pectin degrading enzyme polygalacturonase (PG) that breaks down the cell wall matrix. Within the complete genome of *Arabidopsis*, 69 putative polygalacturonase genes have been identified and these can be grouped into a phylogenetic tree. Isolation of an abscission-related PG from *Brassica napus* (*PGAZBRAN*), allowed us to isolate its homologous gene in *Arabidopsis thaliana* (*PGAZAT*) (Gonzalez-Carranza, *et al*., 2002) and the promoters of both genes were fused to GUS and GFP reporters. Studies of these promoters have identified other sites where polygalacturonases may be expressed (Roberts, *et al*., 2002) and deletion analyses have revealed some domains that maybe involved in regulating the spatial and temporal expression patterns.

Gonzalez-Carranza, *et al*., (2002). *Plant Physiology*. 128: 534-543
Roberts, *et al*., (2002). *Annu. Rev. Plant Biol*. 53: 131-158
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2-19 *cryptic precocious-1D*: a novel semi-dominant mutation that promotes the floral transition

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Flowering-time gene *FT* integrates signals from several genetically distinct pathways that regulate the floral transition. Ectopic over-expression of *FT* causes extremely early-flowering phenotype independent of the activity of these regulatory pathways. The FT protein belongs to an emerging protein family that may represent a new class of evolutionary conserved protein kinase regulators. However, the actual biochemical role of FT protein in the floral transition remains to be elucidated. To gain some clues to understand how *FT* gene regulates the floral transition, we have performed a genetic screen for suppressors and enhancers of *35S::FT* transgenic lines. A semi-dominant enhancer, *cryptic precocious-1D* (*crp-1D*), which eliminated vegetative phase of a weak *5S::FT* line was identified. A semi-dominant effect of the *crp-1D* was specific to the over-expression of *FT*. In non-transgenic background, *crp-1D* showed slightly early-flowering phenotype both in short-day and long-day conditions. We performed map-based cloning and found a nucleotide change causing an amino acid substitution in one candidate gene. A knockout mutant of this gene showed late-flowering phenotype, that is opposite to early-flowering phenotype of semi-dominant *crp-1D.* Characterization of this candidate gene will be presented.

2-20 The *APL* locus is required for phloem development in Arabidopsis roots

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The mechanisms behind vascular development are still largely unknown. We have isolated and partially characterized a novel recessive *Arabidopsis* mutant, *apl* (Altered Phloem Development; previously named *woody*) that has a defect in the organization of phloem poles in the root. *apl* seedlings have a short, determinate root with only occasional lateral branches. Whereas the outer cell layers of the *apl* roots have a normal radial pattern, the vascular system is abnormal. In the *apl* 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. We postulate that APL gene product is required for several aspects of phloem development in the root: (**1**) the specific divisions organizing the phloem pole, (**2**) sieve element differentiation and (**3**) the expression of a companion-specific gene. Here we present the recent progress in the developmental characterization of the apl mutation and molecular cloning of the corresponding gene.

2-21 Large scale identification of senescence related genes

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Leaf senescence is the last stage of development during which cells undergo a major transition from carbon assimilation and other anabolic reactions to a catabolic pattern that results in cell dysfunction, structural disintegration and, eventually, cell death. Biochemical and molecular studies of leaf senescence have provided evidence that this developmental stage is a genetically programmed and active process of self-destruction. Thus, identification of the genes being expressed during senescence and the regulatory mechanisms involved, would provide necessary information for understanding this process. To search for new Senescence Associated Genes (SAGs) and to expand the assortment of SAGs,We have employed the method of suppression subtractive hybridization (SSH), which generates libraries of differentially expressed clones in senescing leaves of Arabidopsis. Several hundreds SAGs have been isolated ,part of them have been analyzed for DNA homology and several dozens of novel SAGs not previously reported to be associated with senescence were found. The functions of the putative proteins indicate potential new biochemical and cellular events that are related to the senescence syndrome. Based on these studies,leaf senescence appears to be a complex pattern of gene expression leading to the concept of the existence of multiple regulatory pathways.

2-22 Functional Analysis of *FLC* homologs *FCL3* and *FCL4*

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FLC-LIKE3 (*FCL3*) and *FCL4* are two closely-related *FLC* homologs located at the bottom of chromosome 5. Transcript abundance of both genes is very low and does not increase in the presence of *FRI*. The conceptual proteins are predicted to be MADS-domain proteins if transcripts are similarly spliced as *FLC*. RT-PCR and EST database analyses indicate that *FCL4* produces at least seven splicing variants that include intron retentions, exon skippings, alternative 3' splice site selection, alternative polyA site selections, or a combination of the above. Intron retention introduces premature stop codons, whereas all other transcripts share the same stop codon, but produce conceptual proteins with internal deletions. Preliminary results indicate that *FCL4* transcripts with intron retention are slightly predominant in early- flowering ecotypes and that intronless transcripts predominate in a late-flowering ecotypes. All *FCL3* transcripts analyzed so far retain introns. We are testing the correlation between intron retention and possible functions of these *FLC* homologs.

2-23 Mutations in the *EARLY IN SHORT DAYS* (*ESD1*) locus suppress the flowering time delay caused by mutations in the autonomous pathway

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Flowering time in Arabidopsis is regulated by photoperiod, being promoted under long days (LD) and inhibited under short days (SD). Information on the genes and pathways that participate in flowering inhibition is still scarce and their interaction with the inductive pathways is almost unknown. We have isolated a set of mutations at the *ESD1* locus, which causes early flowering under non-inductive SD, indicating the involvement of this locus in the repression of flowering under those photoperiods. *esd1* mutants also show moderate elongation of hypocotyl and inflorescence internodes, leaf curling and extra organs in the flower perianth. These phenotypic traits are likely related to a reduced sensitivity to red light. In fact, mutant plants are able to flower under continuous red light conditions, which completely inhibit flowering time in wild type plants. Analyses of double mutants for the *ESD1* locus and different loci of the flowering induction pathways suggest that flowering inhibition mediated by *ESD1* is mainly due to the interaction with components of the autonomous pathway. Using a map-based cloning approach, we have delimited the mutation in a centromeric region of chromosome III spanning three BACS. Overlapping cosmids are being transformed to identify the *ESD1*gene by complementation.

2-24 EBS, a new repressor of flowering in Arabidopsis

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Mutations in the *EARLY BOLTING IN SHORT DAYS* (*EBS*) gene of Arabidopsis cause an acceleration of flowering, especially under non-inductive photoperiods (short days- SD). Genetic analyses have demonstrated that the early flowering phenotype of *ebs* mutants requires both the product of *FT* gene and gibberellic acid (GA) biosynthesis. In addition to early flowering, *ebs* mutants show increased expression of floral organ identity genes such as *AGAMOUS*, *APETALA3* and *PISTILATA* within the flowers. We have identified the *EBS* locus, and the predicted aminoacid sequence of the protein suggests that EBS could be part of a protein complex involved in the repression of gene expression by modulating chromatin structure. Since EBS is likely to act as a transcriptional repressor we have analysed the expression of flowering time genes in *ebs* mutants. These analyses have demonstrated that *FT* gene is prematurely expressed in *ebs* mutants grown under SD, whereas the expression of other genes involved in the control of flowering time and GA biosynthesis is not affected in *ebs* mutants. These results indicate that the repression of flowering by EBS is mediated by its effect on *FT* expression. In addition, we have analysed the effect of *EBS* overexpression on the flowering time of Arabidopsis; as for the loss of function *ebs* mutant alleles, 35S:EBS plants display early flowering phenotype. This phenotype is consistent with the hypothesis that EBS could be part of a protein complex, and the accumulation of EBS product in 35S:EBS lines could disrupt the proper formation or function of the complex. Our progress in understanding the molecular mechanism of EBS function will be discussed.

2-25 The *vernalization independence (vip)* mutations define a class of functionally-related genes involved in flowering as activators of *FLOWERING LOCUS C* (*FLC*)

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The late-flowering, vernalization-responsive habit of many Arabidopsis ecotypes is mediated predominately through repression of the floral program by the *FLOWERING LOCUS C (FLC)* gene. To better understand this repressive mechanism, we have taken a genetic approach to identify novel regulators of *FLC*. We characterized numerous recessive mutations that confer early flowering and loss of *FLC* expression in the absence of a vernalizing cold treatment. A subset of these, designated *vernalization independence (vip)*, collectively define a functionally-related gene class of at least six members in Arabidopsis. Epistasis analysis indicates that the *VIP* genes act in a separate pathway from previously identified *FLC* regulators, including *FRIGIDA* and the autonomous flowering pathway gene *LUMINIDEPENDENS*. Interestingly, *vip* mutants flower even earlier than an *FLC* null mutant, suggesting that *VIP* genes regulate flowering-time genes in addition to *FLC*. We have identified *VIP3* and *VIP4* by positional cloning and T-DNA tagging, respectively. *VIP3* encodes a protein containing structural motifs that suggest involvement in a protein complex. The *VIP4* gene product exhibits similarity to proteins from yeasts, *Drosophila*, and *C. elegans*. Constitutive expression of *VIP3* or *VIP4* cannot overcome the repressive effect of vernalization on *FLC* expression, and, unlike *FLC*, expression of these genes is not downregulated in vernalized plants. Thus, *VIP3* and *VIP4* are not sufficient to activate *FLC*, and are probably not directly involved in a vernalization mechanism. The *VIP* genes also play a role in floral development that is independent of their relationship with FLC and thus have multiple roles in development.

2-26 Analysis of *FVE*: A gene 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 a mutant involved in the AFPP; the late flowering mutant *fve*. *FVE* has been mapped in the middle genomic region of chromosome 2. Using map-based strategies we have isolated this gene. Sequence analyses of 4 alleles and complementation of the *fve* mutant phenotype with a genomic construct have proven that *FVE* encodes AtMSI4. AtMIS4 is a protein related to the mammalian retinoblastoma associated proteins, which might participate in transcriptional repression by histone deacetylase complexes. Currently, we are studying the expression pattern of *FVE* by northern analysis and promoter::GUS fusion. In addition, putative AtMSI4 interacting partners have been identified using two hybrid assays. Furthermore, we have identified another Arabidopsis gene with high homology to *FVE*, named *FVE-2*, for which two T-DNA insertion mutant alleles have been isolated and are under study.

2-27 *FE*: A flowering gene least in the cloning list

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Flowering induction is under the control of several environmental signals coming from temperature and light. Particularly, light plays an important role in the promotion of flowering in Arabidopsis through the length of the light period (photoperiod) since it flowers much earlier when grown under long-day (LD) light conditions than under short days (SD). Classical genetic screenings have identified Arabidopsis mutants showing an altered flowering response to photoperiod length such as *fha*, *gi* and *co* (Koornneef et al., 1991). The molecular and genetic analyses of these mutants has led to the involvement of blue light photoreceptors, putative membrane proteins and transcription factors in this process. To better understand this environmental regulation of flowering we are studying *fe*, one of the classical mutant genes affected in the photoperiod response (Koornneef et al., 1991) whose analysis has been limited because a single mutant allele is currently available. *fe* mutant plants flower later than wild type when grown under LD but, in contrast to other flowering mutants, it did not show any flowering delay when raised under SD. We have pursued the molecular identification of *FE* gene by positional cloning. Thus we have found that the *fe* mutant carried a single nucleotide mutation leading to an aminoacid substitution in a conserved domain of a putative transcription regulator. Further genetic and molecular characterization of *FE* is underway and its current status will be reported.

Reference

Koornneef M, Hanhart CJ, van der Veen JH. (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol Gen Genet. 229: 57-66

3-01 Identification of a potential target gene of the *Polycomb* Group protein MEDEA from *Arabidopsis thaliana*

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In all organisms, the initiation of the zygotic program requires dramatic changes in gene expression, which have to be tightly regulated. Recent investigations by several laboratories have lead to the identification of genes involved in this process in plants. One of these genes, identified by the maternal effect embryo lethal mutant *medea (mea)*, regulates growth during embryogenesis. The *MEA* gene encodes a SET domain *Polycomb* group protein homologous to the Enhancer of zeste protein from Drosophila. The *FERTILISATION INDEPENDENT ENDOSPERM (FIE)* gene, which shows a mutant phenotype similar to mea, also encodes a *Polycomb* group protein with high similarities to the Extra sex combs protein from *Drosophila*. Both proteins interact with each other and most likely regulate together the expression of common target genes. To identify potential target genes for MEA and FIE we hybridised Affymetrix GeneChips with RNA from the *mea* and *fie* mutants. We could identify one gene, which is commonly derepressed in both, *mea* and *fie*. This gene was called *TOM1* (**t**arget **o**f **M**EA 1). *TOM1* codes for transcription factor, which is expressed in the endosperm and the embryo until the transition stage, then the expression is restricted to the chalacal endosperm. However, in the *mea* mutant *TOM1* expression remains high until embryo growth is arrested at the heart stage. We established transgenic lines with enhanced and reduced *TOM1* expression and will show initial results of the characterisation of these lines.

3-02 Auxin and early embryogenesis

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Traditionally, the plant hormone auxin has not been considered as a major player in early Arabidopsis development. However, recently accumulating data suggest that auxin and its transport may have a role in embryogenesis. First indications came from endogenous application of auxin and auxin transport inhibitors to explanted Brassica embryos, which leads to severe patterning defects. In Arabidopsis it was shown that components of auxin signalling such as AXR1, ARF5/MONOPTEROS and members of AUX/IAA family are expressed in very early stages of embryo development. This is also true for both the efflux and influx components of polar auxin transport machinery. Moreover, genetic disruption of auxin response or transport leads to early developmental aberrations. In addition our findings indicate that the auxin signalling machinery in young embryos is functional and capable of eliciting auxin response. We will present novel data indicating developmental importance for auxin response and transport in Arabidopsis embryogenesis and underlining a role of auxin in early development in general.

3-03 "Pattern moving over matter": maintaining the shoot meristem organization

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Quotation of Newman, stated in the sixties, reflects the observation of botanists that the organization of the shoot meristem is reliably maintained although cells continuously leave the meristem and are replaced by new ones. We previously showed that the stem cells are specified by signaling from an underlying organizing center, expressing the *WUSCHEL* gene, and that the size of the stem cell population is dynamically maintained by a regulatory feedback loop between stem cells and organizing center. This signaling circuitry appears to have the potential to act as a self-regulatory system, that is integrated into a larger regulatory network, controlling organ formation from the shoot apex. Here we address the mechanisms of how the organizing center itself is regulated and how specification of stem cells occurs.

3-04 Determination of cell pattern in the Arabidopsis root epidermis

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Multicellular organisms possess diverse cell types that are organized in particular patterns. In our laboratory, we seek to understand how cells acquire their distinct identities and form appropriate patterns within tissues. Our model system is the formation of the root epidermis in Arabidopsis. The root epidermis is a useful tissue because it contains a single cell layer with only two cell types (hair cells and non-hair cells) and it forms rapidly and continuously after seed germination. Furthermore, mutations affecting the formation of one or the other epidermal cell type do not affect plant viability. Interestingly, the hair and non-hair cell types arise in a position-dependent pattern that implies cell-cell interactions are critical for cell fate specification. In our major project, we have identified and analyzed several genes that influence the position-dependent patterning of the epidermal cell types. Some of these genes (e.g. *GLABRA2* and *WEREWOLF*) encode transcription factors important for non-hair cell specification, whereas others (e.g. *CAPRICE*) help define the hair cell type. By studying interactions between these genes, we have found that transcriptional feedback loops between them are important in establishing the cell type pattern. The WEREWOLF gene product is a positive regulator of both *GLABRA2* and *CAPRICE* gene expression. During epidermis development, positional cues bias the expression of the *WEREWOLF* gene and lead to a relatively high level of *CAPRICE* and *GLABRA2* expression in cells located in a particular position (N), which generates the non-hair fate. The truncated MYB encoded by *CAPRICE* mediates a lateral inhibition mechanism to negatively regulate *WEREWOLF*, *GLABRA2*, and *CAPRICE* gene expression in the alternate position (H), which leads to the hair fate. These results provide a molecular genetic framework for understanding the determination of a cell type pattern in plants.

3-05 Axis-dependent development of lateral organs

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Structure of lateral meristems and organs suggests us that their development is dependent on two crossing axes, the ad-ab axis and the lateral axis, although the molecular nature of the hypothetical axes is not known. Our recent studies showed that putative axis controls the expression pattern of genes working in the spatial specification of lateral organs. One of the genes, *PRESSED FLOWER* (*PRS*), is a member of homeobox gene family. The prs mutant lacks two sepals at lateral positions and the marginal cell files of the remained sepals. *PRS* was expressed at the lateral regions of very young flower primordia and in a small number of cells located at the margin of lateral organs, strongly suggesting to be controlled by the putative lateral axes. We observed the *PRS* expression is transiently decreased at the timing that floral meristem becomes to be “independent” from the inflorescence meristem. Another gene, *FILAMENTOUS FLOWER* (*FIL*), a member of the *YABBY/FIL* gene family, is involved in the specification of the abaxial side of lateral organs. *FIL* gene expression was restricted at the abaxial side of the lateral organ primordia, suggesting that *FIL* gene expression is under control of the putative adaxial-abaxial axis in the lateral organ primordia. Promoter analysis of the *FIL* gene suggests the mechanism of the abaxial side-specific expression.

3-06 FOUR LIPS is a MYB protein that regulates cell cycle exit and terminal differentiation in Arabidopsis stomatal development

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Stomata consist of paired guard cells surrounding a pore that allows gas exchange necessary for photosynthesis. Arabidopsis stomata develop through asymmetric divisions of stem cells followed by a symmetric division of the guard mother cell (GMC). The GMC division produces two daughter cells that differentiate terminally as guard cells. Stomata do not contact each other, a patterning process that requires TMM, a receptor-like protein, to orient formative asymmetric divisions (Nadeau and Sack, this meeting). Here we show that patterning also requires a MYB protein encoded by *FOUR LIPS* (*FLP*). FLP is required for the proper number of symmetric divisions, since *flp* mutations result in clusters of laterally aligned stomata. Analysis of the same cells through time shows that *flp* clusters are clonal, with each cluster derived from a single GMC. *flp* GMC daughter cells exhibit ultrastructural features of GMCs and delayed expression of a guard cell-specific promoter::GUS construct. These data show that the *flp* patterning defect results from extra symmetric divisions produced by a reiteration of the GMC program. Thus, FLP is necessary to restrict the number of GMC divisions to one and is a positive regulator of terminal differentiation in the pathway. Transformation of *flp* plants with a highly homologous Arabidopsis MYB gene under its native promoter results in complementation suggesting functional redundancy in the pathway. Homologous genes are present in many plants indicating that the functions of these MYB proteins are conserved.

3-07 The *KNAT6* homeobox gene of *Arabidopsis thaliana* is expressed in roots

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Homeobox genes encode transcription factors that contain a highly conserved DNA binding domain within their DNA sequence. In animals, these genes are involved in body plan organisation and patterning. In plants, their role is less clear cut and they appear to have additional roles in other developmental and response processes.
mRNA from Arabidopsis roots was used as a template for 3' Random Amplification of cDNA Ends (RACE) using degenerate primers designed to the *KN1* family of plant homeobox genes. A novel transcript was isolated and has been designated *KNAT6*.
Promoter-GUS analysis has revealed that this gene is expressed in the roots of seedlings as well as in some aerial parts such as floral organs and leaves. The regulation of gene expression throughout development and the response of gene expression to various plant hormones is being investigated using these GUS transgenic lines.
In addition, downregulation of gene expression using RNAi and antisense, as well as overexpressing lines, are being used to gain information on the function of this gene in Arabidopsis. Several putative T-DNA knockout lines have also been obtained and are being analysed.

3-08 The *IRE* gene encodes a protein kinase homologue and modulates root hair growth in Arabidopsis

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We have studied the developmental processes of root hairs using a molecular genetic approach. We screened T-DNA insertion lines of Arabidopsis for mutants with abnormal root hair formation. Two T-DNA tagged mutants that showed abnormal root hair lengths were isolated. One of them, named *incomplete root hair elongation* (*ire*), has root hairs about 40% shorter than the wild type owing to the early cessation of their growth. In contrast, the other, an allele of *hy5*, has root hairs about twice as long as the wild type, possibly because of delayed cessation of growth. Thus *IRE* and *HY5* are likely to modify the duration of growth of root hairs. The *IRE* locus encodes a protein that includes a serine/threonine protein kinase domain. The primary structure of this kinase domain shows significant similarity to a group of protein kinases among various eukaryotes. In the Arabidopsis genome there are at least three genes that are closely related to *IRE*. The IRE transcript was detected in every organ examined. However, the *IRE* promoter-GUS fusion gene was strongly expressed in the elongating root hair cells, suggesting the cell-autonomous function of IRE in root hairs. GUS activity was also detected in pollen grains, which develop by tip growth, suggesting that *IRE* has a common role in the tip growth of plant cells.

3-09 Characterization of *POM2*, a gene involved in regulating cell expansion in *Arabidopsis thaliana*

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Cell expansion is an essential parameter for plant morphogenesis. In screens of root morphogenesis mutants four *pom2* alleles have been isolated which develop short fat roots. Moreover hypocotyl elongation under skotomophogenic growth conditions is affected as well. *pom2-1* and *pom2-2* exhibit a reduced apical dominance and are nearly sterile. These alleles have smaller flowers with shorter stamens and gynoecia. Fertilized flowers develop stunted siliques with only few seeds.
The *POM2* gene was mapped to the center of chromosome II. Analyzing more than 1000 chromosomes, *POM2* was positioned within a 110 kb interval containing 36 candidate genes. Using a non-radioactive labeling method for whole BAC clones allele specific polymorphisms in two *pom2* alleles was detected. Restriction map analyses located all these polymorphisms to one region where a gene coding a 2048 amino acid protein of unknown function is annotated. Mutations in all four alleles were identified by sequencing. The two fertility reduced alleles *pom2-1* and *pom2-2* have both deletions in the first intron and are most probably null alleles. *pom2-3* has a single base pair deletion in exon II leading to a frameshift and a premature stop codon. *pom2-4* has a 2446 bp deletion starting in exon III and ending in the adjacent gene. We will present data on the phenotypic characterization of the four *pom2* alleles and the cloning of the *POM2* gene.

3-10 Functional analysis of *PLEIADE*, a key player in cytokinesis of *Arabidopsis thaliana*

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In a screen for root morphogenesis mutants in *Arabidopsis thaliana* three *pleiade (ple)* alleles were identified, exhibiting a number of features characteristic for cytokinesis defects. The *ple* mutants have shortened, irregular expanded roots and enlarged multinucleated cells with cell wall stubs, while the aerial parts are not affected. The *PLE* gene was cloned by a map-based approach and consists of 12 exons coding for a 707 aa protein. Related proteins are present in higher eukaryotes as mouse and humans. *PLE* belongs to a small gene family of nine members in *A. th.*, spread over all 5 chromosomes. Expression of these members, analyzed by real-time PCR, shows overlapping organ specificity. The predicted PLE protein contains two putative CDK and seven putative MAPK phosphorylation sites. One of these kinases may be responsible for phosphorylation and thus for posttranslational regulation of PLE. Data on the functional relevance of the putative phosphorylation sites will be presented.

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3-11 Role of *TCP* genes in axillary meristem development

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The TCP genes code for putative transcription factors containing the so-called "TCP domain" a predicted non-canonical bHLH region that mediates DNA binding and protein-protein interactions. The best-characterised members of this family are *TEOSINTE BRANCHED 1* (*TB1*), responsible for the apical dominance in maize and *CYCLOIDEA* (*CYC*) that controls the dorsoventral asymmetry of *Antirrhinum majus* flowers. Subtle changes in the expression patterns and levels of these genes have been responsible for two important evolutionary events: maize domestication (*TB1*) and the evolution of floral asymmetry in angiosperms (*CYC*).
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*, transcription factors from rice.
We are particularly interested in the *TB1 CYC* subfamily. Expression pattern studies show that *TCP1* (ortholog of *CYC*) and *TCP12* and *TCP18* (orthologs of *TB1*) are expressed in axillary meristems similarly to their counterparts in *Antirrhinum* and maize. We have constructed RNAi lines for these TCP genes and their mutant phenotypes suggest that these genes play an important role in axillary meristem development. Our results will be presented.

3-12 Seed germination: The role of PROHIBITIN

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We are interested in the mechanisms that control germination: gene expression and early cell differentiation during this process. In a previous AFLP experiment (de Diego et al., *Plant Molecular Biology Reporter* 8:2 Supplement S18-2, 2000) designed to characterize cDNA fragments corresponding to mRNAs induced during *Arabidopsis thaliana* seed germination, we isolated a prohibitin gene differentially expressed during germination in wild type seeds compared to *abc33 (ga1)* mutants. We will present data concerning water uptake as well as expression of a prohibitin gene in *Arabidopsis thaliana* seeds during germination. We have found differences in water uptake among diverse genotypes affected in the ethylene signal transduction pathway (*eto, ein, etr*) when compared with the wild-type cv. Columbia. Northern blot indicates differential expression of prohibitin gene in ethylene mutants as well as GA1 mutant. This finding is in contrast with a Western blot experiment which shows the presence of prohibitin in all the mutants analysed, as well as in the wild type at different times after imbibition. Prohibitin in yeast and mammalian cells plays an important role in the control of cell cycle progression through the binding to Retinoblastoma protein. Moreover, binding to E2F and raf kinases has also been described. These results point to new mechanisms of control of seed germination by which hormones may interact with cell cycle components.

3-13 Does a CLV-like signaling pathway control balanced cell division and differentiation in the root mereistem?

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Plant development continues after embryogenesis through the activity of small groups of continuously dividing cells, the meristems. Meristems generate the majority of plant organs through balanced cell proliferation and differentiation. Within the root meristem of *Arabidopsis thaliana*, four mitotically inactive cells, the quiescent center (QC), are surrounded by the stem cells responsible for continuous formation of new root cells.
We are interested in genes involved in the balance of cell division and differentiation in the root. Overexpression of a *CLV3* like gene (*LLP1*) under a root meristem specific promoter, causes root meristem differentiation, suggesting that a *CLV* like pathway is responsible for root meristem maintenance. To identify the genes involved in these signaling pathway we performed an EMS mutagenesis suppressor screen on the *LLP1* overexpressor line. Several suppressors have been identified, and a map based cloning approach is being used to identify the corresponding genes.

3-14 Isolation of mutants that suppress the effects of ectopic *SHOOT MERISTEMLESS* activity

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The *SHOOT MERISTEMLESS (STM)* gene is necessary to initiate and maintain a shoot apical meristem. In the central region of the meristem the gene maintains the undifferentiated cell state and in the peripheral region *STM* is downregulated in areas of primordia development.
To investigate *STM* controlled genes and functions, we generated plants with ectopic *STM* activity. Ectopic expression of STM causes the arrest of the seedling development, so a steroid inducible system has been used in which STM is fused to GR (rat glucocorticoid receptor).
In plants with ectopic STM activity, development of leaf primordia is inhibited once they begin to protrude from the meristem. In arrested primordia, cell expansion and morphological signs of cell specialisation were negligible. STMGR was crossed to cell cycle markers for cyclin D (to monitor transition from G1 phase to synthesis phase) and cyclin B (to monitor progression from G2 to mitosis). Ectopic STM activity seemed to inhibit cell differentiation without significantly activating cell division.
To identify additional genes that act in the STM pathway, mutations that suppress the effects of ectopic STM were generated. The STMGR seeds were mutagenised with EMS and the F2 plated on medium containig dexamethasone, causing ectopic STM activity and therefore the arrest of the seedlings. Nine were found that suppressed the STMGR effect. The mutants are now being characterised and mapped.

3-15 Overexpression of *FRC2*, a katanin-like protein

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Arabidopsis trichomes are an excellent model in which to study cell shape determination. Using this system, the *frc2* mutation was isolated in a screen to identify genes involved in trichome branch initiation. Mutations in *FRC2* result in trichomes with two and three branches. *FRC2* has been shown to be allelic to *FRA2*, which encodes a katanin–like protein (Burk *et al*., 2001).
Katanins have been extensively studied in animal model systems. They are regulatory proteins responsible for ATPase dependent microtubule severing. Biochemical analysis of animal katanin reveals it is a heterodimer, composed of 60 and 80 KD subunits. The 80 KD subunit is believed to target the katanin to the centrosome in animals, while the 60 KD subunit has been attributed with the actual severing of microtubules. Their most prominent function in animal cells is in severing of microtubules during mitosis and meiosis. However, the *FRA2/FRC2* katanin is not important for microtubule severing during cell division, but is required for interphase severing in the production of normal cell wall biosynthesis and cell expansion (Burk *et al*., 2001).
Genetic evidence suggests that *FRC2* is partially redundant to *ZWI*. To directly test this hypothesis, we PCR amplified the *FRC2* gene from a genomic clone provided by Dr. Z.H. Ye (University of Georgia) and overexpressed it in a *zwi* mutant background, as well as other mutant backgrounds. Overexpression was accomplished using the estrodiol-inducible pER8 system (Zuo *et al*., 2000). We expect overexpression of *FRC2* in most of these backgrounds to rescue the mutant phenotype.

Burk D.H., Liu, B., Zhong, R. Morrison, W.H., and Ye, Z.H. (2001). A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. Plant Cell **13**: 807-27.
Zou, J., Niu, Q.-W., and Chua, N.-H. (2000). An estrogen receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants. Plant Journal **24**: 265-273.

3-16 Towards the functional characterization of the ARIADNE-RING-finger gene family in *Arabidopsis thaliana*

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RING-finger-proteins were identified in a wide rage of eukaryotic organisms and are involved in diverse cellular functions. They are classified due to their combination with other protein motifs. Members of the ARIADNE protein gene family are characterized by the present of a N-terminal acid-rich cluster, followed by a C3HC4 RING-finger motif, a central cysteine-rich in between RING (IBR) region or B-box and RING-finger-like structures. At the C-terminus these proteins have a potential coiled-coil domain designating them as the ARBRCC (acid-RING-B-box-RING-coiled-coil) subgroup of RING-finger proteins. They belong to a conserved eukaryotic protein family found in *D. melanogaster*, *S. cerevisiae*, *C. elegans*, *M. musculus* and *H. sapiens*. It has been shown that ARIADNE proteins are interacting with specific E2 ubiquitin-conjugating enzymes thus may play an essential role in ubiquitin mediated protein degradation.
In *Arabidopsis thaliana* we identified 16 genes which are closely related to the *ARIADNE* class. Phylogenetic analysis divides them into three distinct subgroups with up to eight members. To determine if all genes of the family are expressed, we measure the level of expression in different organs using real-time quantitative PCR. The differential expression pattern portends overlapping and specialize roles in different organs. To address the function of this gene family we initiated a reverse genetic approach by screening for "knock out" and "enhancer trap" alleles. The phenotypic characterization of six single mutants and the progress on double mutant analyses will be presented.

3-17 Preliminary analysis of RNA extracted from Arabidopsis embryos by laser capture microdissection

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The basic body plan of the Arabidopsis seedling is established during embryogenesis. Although the histology of this process has been well characterised, as yet we have only a limited understanding of the genes and transcriptional changes required for the establishment of embryo pattern, morphogenesis and cell differentiation. Microarray analysis has greatly facilitated our ability to examine changes in transcriptomes, but the small size and poor accessibility of Arabidopsis embryos has made such analysis difficult. Furthermore, due to these technical difficulties, analysis of specific zones or cell types of young embryos has been almost impossible. Laser Capture Microdissection (LCM) has simplified the problem of separating specific cells from complex tissues. In essence, LCM involves cutting histological sections of the tissue of interest and then targeting the cells of interest with a laser, allowing for their removal from the surrounding tissue. RNA can then be purified from these cells and subjected to analysis. It is our aim to use LCM to examine transcriptome changes between different zones of the embryo. Preliminary RT-PCR data from RNA extracted from LCM cells of Arabidopsis embryos will be presented.

3-18 Hypocotyl cells of the *turnip* mutant of Arabidopsis undergo cellular redifferentiation

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A second site regulator screen of the *polaris* mutant of Arabidopsis, defective in auxin-cytokinin homeostasis (Casson et al., *Plant Cell* in press), led to the identification of the *turnip* mutant. This is characterised by the development of an unusually dense cellular structure at a swollen root-hypocotyl junction. The radial pattern of the structure is similar to that of WT hypocotyl, with an epidermis, two cortical layers, endodermis, pericycle and vascular bundle. However, unusual cell divisions and expansion are occasionally observed. The cells are avacuolar and are packed with starch granules. SEM analysis shows that the epidermal cells of the structure are significantly smaller than those of the *pls* parent. However, at the boundary of the structure, epidermal cells undergo a large degree of apical-basal expansion. Cells in the structure fail to show expression of epidermal and endodermal hypocotyl markers, and ectopic expression is observed of genes not normally expressed in the hypocotyl. These data indicate that the hypocotyl cells have undergone changes in cellular differentiation. The *turnip* phenotype is not fully penetrant, and sucrose, but not glucose or fructose, increases penetrance. The phenotype is also not light-dependent when grown in the presence of sucrose. Interestingly, dark grown *turnip* seedlings show some light-grown characteristics with formation of first leaves and root branching. Penetrance of the *turnip* phenotype is also affected by hormones – exogenously applied auxin and auxin transport inhibitors each led to increased penetrance, whereas exogenous cytokinin was found to reduce penetrance.

3-19 Combined *SHOOT MERISTEMLESS* and *WUSCHEL* trigger ectopic organogenesis in Arabidopsis

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Aerial development of plants happens through the generation of new organs at the shoot apical meristem. Both *SHOOT MERISTEMLESS* (*STM*) and *WUSCHEL* (*WUS*) genes have been shown to play a crucial role in this development: it has been proposed that the role of *STM* is to retain the cells undifferentiated in the shoot apical meristem whereas *WUS* keeps a constant pool of cells available in the central zone of the shoot apical meristem. We present elsewhere an inducible system allowing *STM* expression throughout the plant. Although the ectopic expression of *STM* can trigger the expression of some genes involved in meristem development and /or cell division machinery, it was not sufficient to induce ectopic meristem formation (See Woodward et al.).
We set up a mosaic expression of *WUS* in Arabidopsis and showed that, by combining both expressions of *WUS* and *STM*, we induced ectopic cell division and organogenesis leading to organ primordia formation on both cotyledons and hypocotyls. These primordia derive from cells with meristematic activities including the expression of the central zone marker *CLAVATA1*. We showed that *WUS* is not expressed in these primordia and therefore acts non-cell autonomously, as it is the case in the shoot apical meristem.
Our results suggest that *STM* and *WUS* interact non-additively to trigger cell division and establish meristem activity.

3-20 Functional analysis of the Arabidopsis *TFM-S* gene

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In the attempt of elucidating the functional role of the MYB-LIKE Trancription Factor-S (TFM-S) we have analyzed the expression profile of the wild type allele in different plant tissues by RT-PCR and in-situ hybridization. Data indicate that TFM-S is expressed in the epidermal and sub-epidermal layers of leaves, sepals and carpels.
To further characterize the expression of TFM-S we have cloned a 900bp upstream region in front of the reporter gene GFP. Transgenic plant expressing the fusion construct exhibit a strong GFP expression in the guard cell of the stomata present in cotyledons, rosette leaves, stems, sepals and carpels.
Recently, we have identified, by a PCR-based screening approach, a null allele of TFM-S, in the Yale University T-DNA insertion lines. Plants homozygous for the insertion closely resemble the phenotype of ttg1 and ttg2 mutants. Mutant TFM-S individuals exhibit a glabrous phenotype in the rosette leaves, stems and sepals. Abnormal trichomes are exceptionally found only at the leaf margin. Moreover, mutant seeds lack the characteristic purple anthocyanin pigment in the endothelium layer and do not produce mucilage upon imbibition.
All together, these preliminary observations, suggest a role for the wild type TFM-S gene product in controlling different metabolic pathways confined to the epidermal and sub-epidermal layers of Arabidopsis organs.

3-21 The *TUMOROUS SHOOT DEVELOPMENT 1 (TSD1)* gene alters cytokinin-sensitivity and is required for co-ordinated plant development

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Plants of *Arabidopsis thaliana* mutated in the *TSD1 (TUMOROUS SHOOT DEVELOPMENT 1)* gene develop mainly unorganized growing tissue instead of organized stem and leaves. The resulting green callus can be cultivated in vitro on medium without auxin and cytokinin. *tsd1* mutants react to exogenous cytokinin with an enhanced growth response. The steady state transcript levels of the *CKI1* gene and meristem specifying homeobox genes are increased in the mutant. Histological analyses showed that the SAM of *tsd1* mutants forms only rudimentary leaves and its L1 cell layer degenerates several days after germination. The *tsd1* mutation is recessive and maps to the bottom of chromosome 5. The *TSD1* gene was isolated by map-based cloning.

3-22 Functional characterization of the Clp protease complex in Arabidopsis plastids through reverse genetics and proteomics

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Arabidopsis chloroplasts are predicted to contain up to 2500-3000 proteins. Protease activity in the plastid is essential for protein housekeeping and stoichiometry, regulation of plastid gene expression, as well as processing. However, the structures and functions of the numerous predicted chloroplast-localized proteases are poorly understood.
Using non-denaturing gels and mass spectrometry, we have recently identified a chloroplast localized 350 kDa protease complex in Arabidopsis, composed of 11 ClpP,R,S subunits (Peltier et al. 2001, JBC 276, 16318-16327). The Arabidopsis nuclear genome contains at least 26 Clp-related genes, and we predict that 15 of these are plastid-localized. An additional ClpP protein is plastid encoded and is essential in tobacco and *C. reinhardtii*. Clp proteins (for **c**aseino **l**ytic **p**rotease) were first identified in *E. coli* as an ATP-dependent serine type protease complex, organized in two identical homo-heptameric rings of ClpP subunits.
The aim of this project is to use reverse genetics, functional complementation with epitope tagged Clp genes and proteomics to address the functional complexity of the Clp protease in *Arabidopsis thaliana*.
We screened an Arabidopsis T-DNA insertion collection and, under optimal growth conditions, we identified several Clp mutants homozygotes lethal. For other Clp genes, we did isolated homozygotes lines and we will present data on the genotypes and phenotypes, as well as the Clp complex composition of these homozygous Clp mutants.

3-23 TTG2, a WRKY transcription factor of Arabidopsis, is involved in trichome development and in mucilage and tannin production in the seed coat

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In Arabidopsis, mutants of *TRANSPARENT TESTA GLABRA2* (*TTG2*) show reduced numbers of leaf hairs that are unbranched. Also seed coat mucilage is absent, and tannins do not accumulate in the endothelium. These multiple roles overlap in part with those of TTG1. The *TTG2* gene was tagged by *Tag1*and shown to encode a WRKY transcription factor (WRKY44). In trichome development, TTG2 apparently acts downstream of TTG1 and GLABROUS1, but it shares functions with GLABRA2. In seed coat development, TTG2 requires TTG1 function for tannin production. *TTG2* is also expressed strongly in the atrichoblasts of roots, but these are affected in *ttg2* mutants, and this expression is not dependent upon TTG1 or GL2 function. Unlike other WRKY genes tested, *TTG2* expression is not stimulated by salicylic acid, wounding or aging. This suggests that its function has diverged markedly from other members of this large family, and that it now regulates the expression of genes involved in the differentiation of at least three different cell types derived from L1 cells.

3-24 Genes that balance division and differentiation in the root meristem

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Plant development continues after embryogenesis through the activity of small groups of dividing cells, the meristems. Meristems generate the majority of plant organs through balanced cell proliferation and differentiation. To determine how the rate of cell division and differentiation can be coordinated and what role patterning genes have in this, we overexpressed genes of the basic cell cycle machinery in different places in the root meristem. This overexpression did not change meristem morphology. Activity of the meristematic tissue in wildtype *Arabidopsis thaliana* plants is regulated by the prescence of the quiescent center (QC) but also by the lateral root cap and columella. Genetic ablations of columella and lateral root cap, by expressing diphtheria toxin A-chain in root cap cells in transgenic "DiphT" lines, lowered meristematic activity severely (Tsugeki and Fedoroff, 1999). To identify the targets of putative signals from the lateral root cap or columella involved in maintaining meristematic activity we performed a supressor screen on the "DiphT" line, using EMS. Two unlinked supressors were recovered, where root meristem activity and growth were restored, although plants still lacked a columella and lateral root cap. Currently we are cloning these two genes and analyzing their roles in maintaining meristematic balance.

Tsugeki and Fedoroff (1999) PNAS 96, 12941-12946

3-25 Functional analysis of *PLS* in root development

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To identify novel genes that regulate root growth and development, a strategy of promoter trapping was carried out on *Arabidopsis thaliana*. One such gene identified in this screen is *POLARIS* (*PLS*). *PLS* has been cloned and encodes a predicted polypeptide of 36 amino acid residues. *PLS-GUS* fusion activity is first detectable during embryogenesis, from the early heart stage when the root meristem is being constructed and predominately in the seedling root tip with low expression in the aerial parts. The *pls* mutant exhibits a semi-dominant short root phenotype and reduced leaf vascularization (Casson *et al*., 2002: *Plant Cell*, in press). Studies have demonstrated that *PLS* is expressed, not as a feature of cell differentiation *per se* during root tip construction, but in response to cell position that is independent of cell type at the root tip. A model has therefore been proposed in which *PLS* represents a component of a signalling pathway that defines positional information in the embryonic root. We are interested in both the role of the *PLS* gene and the nature of the signals that are required for the observed spatially restricted pattern of *PLS* expression which may define at least some aspects of polarity in the Arabidopsis root. Evidence is presented that *PLS* is required for correct auxin-cytokinin-ethylene homeostasis to modulate root growth and vascular patterning.

3-26 *CUC1* promotes adventitious SAM formation on cotyledon through *STM* induction

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The shoot apical meristem (SAM) is formed during embryogenesis and functions to continuously generate the above ground parts of plant after germination. *CUC1* (*CUP-SHAPED COTYLEDON1*) and *CUC2*, encoding members of the *NAC* family, are functionally redundant genes that are involved in SAM formation and cotyledon separation during embryogenesis. We examined the transgenic plants overexpressing *CUC1* under the control of the CaMV 35S promoter (*35S::CUC1*). Cotyledons of *35S::CUC1* seedlings had two severe lobes, small and round epidermal cells between the lobes, and adventitious SAMs on the adaxial side of these cells, suggesting that *CUC1* promotes adventitious SAM formation by maintaining some epidermal cells to be undifferentiated. It has been shown that the *STM*, *KNAT1*, *CUC2* and *WUS* genes are required for the SAM formation and/or maintenance. In *35S::CUC1* cotyledons, both *STM* and *KNAT1* were ectopically expressed before adventitious SAM formation, but *CUC2* and *WUS* were not. *STM* was ectopically expressed during late embryogenesis on cotyledon primordia, while *KNAT1* was ectopically expressed on cotyledons after germination. The expression patterns of *STM* and *KNAT1* were partially overlapped on *35S::CUC1* cotyledons. To confirm whether these class I *knox* genes are essential for adventitious SAM formation, *35S::CUC1* transgene was integrated into *stm* and *bp/knat1* mutant backgrounds. In the *stm*, ectopic expression of *CUC1* could not induce adventitious SAMs, whereas it could induce adventitious SAMs in the *bp*. These results suggest that *STM*, but not *KNAT1*, is necessary for adventitious SAM formation in *35S::CUC1*.

3-27 The *BODENLOS/IAA12* gene mediates auxin response during Arabidopsis embryogenesis

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Auxin has been implicated in various aspects of plant development, including organ formation during embryogenesis. Response to auxin is thought to be mediated by interacting pairs from two gene families, the AUX/IAA family of transcriptional repressors and the ARF transcription factor family. Loss-of-function mutations in the *MONOPTEROS (MP)/ARF5* gene cause a deletion of basal seedling structures. A mutation in the *BODENLOS (BDL)* gene leads to similar seedling phenotypes and the mutant seedlings are resistant to 2,4 D (an auxin analogue). Genetic experiments have shown that *BDL* interacts with two genes involved in auxin response, *MP/ARF5* and *AUXIN RESISTANT 1 (AXR1)*. Furthermore, the early-embryo phenotypes of *mp* and *bdl* are very similar, if not identical.
Here we show that the *bdl* mutant phenotype is caused by a gain-of-function mutation in the *IAA12* gene putatively stabilising the protein. The gene is expressed during embryogenesis from an early stage on in a very dynamic fashion similar to the *MP* expression pattern and both proteins interact in yeast. The molecular and the genetic data support the hypothesis that *BDL* and *MP* form a pair of IAA/ARF genes mediating auxin response during formation of basal seedling structures.

3-28 Trehalose metabolism and Arabidopsis embryogenesis

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Trehalose, a common disaccharide, was thought absent in plant carbon metabolism, except in that of some specialised desert species. Cloning of Arabidopsis genes by complementation of defective yeasts, however, proved that Arabidopsis expresses enzymes capable of trehalose metabolism such as the AtTPS1, a trehalose-6-phosphate synthase; AtTPP-A and AtTPP-B, trehalose-6-phosphate phosphatases and trehalase (AtTRE1). We are studying the role of trehalose metabolism in Arabidopsis using the genetic knockout- approach.
The Arabidopsis *tps1-2* mutant has a transposon inserted in the first exon of *AtTPS1*. The mutation is recessive with heterozygous plants appearing normal. Homozygous mutant seeds are wrinkled and contain embryos arrested at the seed filling stage of development. Already during body pattern formation the mutant embryonic development is retarded in growth and eventually stops at torpedo stage, resulting in non-viable seeds. Complementation of *tps1-2* with the wild type *AtTPS1* sequence rescues the observed defects fully indicating that a functional *AtTPS1* gene is essential for development of the Arabidopsis embryo. OtsA, the E.coli TPS1 homologue, fused to the AtTPS1 promoter, can also rescue the embryonic defect. This rescue is probably due to the presence of the enzyme product trehalose-6-phosphate, which therefore may play a critical role during Arabidopsis embryogenesis. In conclusion we have demonstrated that a component of the trehalose biosynthetic pathway is essential for Arabidopsis embryo development. In order to study the role of AtTPS1 throughout plant development, we are currently using a hormone inducible *AtTPS1* expression system to enable *tps1-2* mutant rescue into viable seed.

3-29 Regulation of *CLAVATA3* (*CLV3*) expression in *Arabidopsis thaliana*

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The *CLV3* gene controls the maintenance of stem cells in the shoot apical and floral meristems of *Arabidopsis thaliana*. *CLV3*, which encodes a small protein that is secreted from the tip of aboveground meristems, is able to bind to a heterodimeric receptor complex, consisting of CLV1 and CLV2. The *CLV1* and *CLV2* genes encode LRR kinase-like transmembrane protein with or without a cytoplasmic signalling domain, respectively, and activation of the CLV1/CLV2 complex by CLV3 results in repression of *WUSCHEL* expression. The WUSCHEL homedomain protein is expressed beneath the stem cell zone and promotes stem cell identity and *CLV3* expression at the meristem tip. Thus, the mutual regulation between *CLV3* and *WUS* controls the balance between pluripotential stem cells and differentiated cells, which give rise to lateral organ primordia.
We are currently investigating two aspects of the regulation of CLV3:
1. We are using a new technique for clonal analysis to test whether all CLV3 expressing cells are stem cells. 2. We have started to dissect the *CLV3* promoter and have identified elements that are required for the specific expression pattern and the *WUS* dependent upregulation of *CLV3*. Further analysis via a yeast one-hybrid system is in progress to isolate genes that control *CLV3* expression.

3-30 Internal telomeric repeats and "TCP" domain protein binding sites cooperate to regulate gene expression in *Arabidopsis thaliana* cycling cells

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Regulation of gene expression is a key step for G1/S transition of the cell cycle. We have focused our interest on two cis-regulatory elements, named site II motif and telo box respectively, identified within the promoter of plant *Proliferating Cellular Nuclear Antigen (PCNA)* and putatively involved in meristematic expression of the gene. A conserved topological association between site II motif and telo boxes is observed in the promoter of numerous genes expressed at the G1/S transition, including several cell cycle related genes and 153 genes encoding ribosomal proteins. Meristematic expression of a GUS reporter gene was observed in plants under the control of the Arabidopsis site II motif within a minimal promoter. This expression is strongly enhanced by addition of a telo box within this chimaeric promoter. We showed by gel retardation experiments that the site II motif can bind a transcription factor, At-TCP11, from the TCP-domain protein family. At-TCP11 has been identified by a yeast two hybrid approach as a putative element in the signalling pathway of the "lectin" receptor kinase lecRK-a1. A recombinant At-TCP11 protein is phosphorylated *in vitro* by the kinase domain of lecRK-a1. Moreover At-TCP11 appears to be a potential partner of Atpura which was previously shown to bind telo boxes. Together these results suggest:

(I) The implication of TCP domain proteins in development linked to meristem activity.
(II) The importance of internal telomeric sequences to regulate such process.
(III) A putative role for lecRK-a1 in the perception and the transduction of mitogenic signals.

3-31 Large-scale functional analysis of genes required for early embryo and endosperm development in Arabidopsis

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The plant seed is a complex arrangement of maternal tissues and of the two products of double-fertilisation, the embryo and the endosperm. Seed development is governed by maternal and zygotic controls involving a large number of genes. Despite the large number of genes essential for seed development, only a subset has been characterised at the molecular/biochemical levels. We have used a systematic approach to obtain insight in a rapid and efficient manner as to the function of EMB genes. The screening for embryo-defective mutations among the Versailles collection has yielded several hundred of mutant lines. Segregation tests on the progeny are performed to determine the linkage of the mutation with the T-DNA. In case of linkage, the FST (flanking sequence tag) is determined. At the end of this research program, funded by Génoplante, more than a hundred FST, putative molecular marker of EMB genes will be available. We present an overview of the screen with details of the efficiency of the mutagenesis and the classes of genes identified by the FSTs.

3-32 Siamese, a regulator of the endoreduplication cell cycle during trichome development resulting 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, DNA is replicated without cellular or nuclear division. One Arabidopsis cell type exhibiting endoreplication is the trichome (leaf hair). Recessive siamese (*sim*) mutations result in multicellular trichomes in place of the unicellular trichomes produced by wild-type plants. Trichomes consisting of up to 15 cells have been observed. Individual nuclei of a multicellular trichome have a reduced level of endoreduplication, indicating that wild-type *SIM* may function to suppress mitosis during the switch to the endo cell cycle. Consistent with this hypothesis, *sim* mutants ectopically express a CYCLIN B1;2::GUS reporter in developing trichomes. 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 identified, including both phenotypic supressors and enhancers. These new mutants are currently being characterized. Progress towards isolating the *SIM* gene will be reported.

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3-33 The *FEZ* and *SOMBRERO* genes are required for lateral root cap (LRC) differentiation in *Arabidopsis thaliana*

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Lateral root cap layers arise from periclinal divisions of the epidermal stem cell/initial. The daughters of such divisions divide horizontally a few times and differentiate. Specific genes are expressed in this region of the root cap, which are not expressed in the distal part of the cap. The first layer of lateral root cap is formed in the embryo where it marks the onset of root meristem activity, and layers are continuously added later on. The outermost layer of the lateral root cap is programmed to slough off from the main root body, at the same rate by which new layers are added by the stem cells/initials.
To identify specific genes required for LRC-differentiation, we performed a genetic screen for changes in the expression of two combined lateral-root-cap-expressed enhancer trap lines: J1092 (GFP, http://www.plantsci.cam.ac.uk/Haseloff/IndexCatalogue.html) and ET244 (GUS, J.E. Malamy and P.N. Benfey, Development 124, 33-44 (1997)). The screen resulted in the isolation of mutations in two genes, *FEZ* and *SOMBRERO (SMB)*. Neither of the mutants completely abolishes LRC specification, but mutants have distinct early LRC differentiation defects, and periclinal devisions are mis-regulated. We have investigated in some detail the phenotypic defects in *fez* and *smb* mutants and we are in the process of mapping and cloning both genes.

3-34 Regulation of *CLV3* expression by *WUS* and *STM*

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In Arabidopsis, the size of the stem cell domain in meristems is regulated by two antagonistic activities: The *WUSCHEL* gene (*WUS*), encoding a homeodomain protein, promotes the formation and maintenance of stem cells. These stem cells express *CLAVATA3* (*CLV3*), and signaling of CLV3 through the CLV1/CLV2 receptor complex restricts *WUS* activity. We have analysed whether expression of *CLV3* is controlled by the activity of *WUS* or another homeobox gene, *SHOOT MERISTEMLESS* (*STM*), that is required for stem cell maintenance. We found that expression of *CLV3* depends on *WUS* function only in the embryonic shoot meristem. At later developmental stages, *WUS* promotes the level of *CLV3* expression, together with *STM*. In the wildtype, a small group of cells underlying the stem cell domain expresses both *WUS* and *STM*. However, *CLV3* RNA is not found in these cells, but in a separate domain at the tip of the meristem. The spatial separation of the *WUS* expression domain from the stem cell domain expressing *CLV3* suggests that either the WUS protein itself, or a WUS-dependent signal is transmitted to the cells at the apex of the meristem. We tried to circumvent a non-cellautonomous function of WUS by expressing *WUS* directly in the stem cell domain from the *CLV3* promoter. Our results suggest that non-cell autonomy of WUS is not a prerequisite for *CLV3* activation in the meristem.

3-35 Promoter analysis of a MYB transcription factor from Arabidopsis

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In vertebrates MYB proteins form a small family with a central role in controlling cellular proliferation, cell cycle progression and differentiation, while higher plants contain a large number of *MYB* genes with mainly unknown functions. Despite single- and three-repeat MYB proteins have been found, the R2R3-MYB subfamily remains predominant in plants. In Arabidopsis, more than 125 *R2R3-MYB* genes have been identified, representing one of the widest family of plant transcription factors described. The information available on the function of a few plant MYB proteins suggest an important role of this family in various processes like: the regulation of metabolic pathways, the control of cell division and of plant morphogenesis, the response of plants to different stresses and in hormone signal transduction.
One of the *MYB* genes under study in our laboratory is expressed throughout flower development and in 4 days-old seedlings, where the activation is mediated by light. Furthermore, in 4 days-old seedlings the transcript is mainly present in the vegetative meristem and in cotyledons, while during flower development the transcript is present in the inflorescence meristem and floral apex, in all flower bud except sepals, in ovule primordia, mature ovules, ovary wall and in the epidermis of embryos. Aim of the present work is the exact mapping of the full-length promoter and the identification of the cis-regulatory element which drives the expression of this gene in a tissue-specific and developmental-specific manner within the embryo epidermis and /or the flower.

3-36 Molecular genetic dissection of the postgenital organ fusion process

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The epidermis of plants provides a barrier against water loss and mechanical damage and normally prevents cell interactions during the early stages of development. Epidermal cells are capable of cell interactions on the adaxial side of the carpels, which join to shape the pistil of the flower. In some mutants, the epidermal barrier is impaired causing ectopic epidermal cell interactions that can result in visible fusions of normally independant organs. Previously, we have described the transposon tagging and cloning of *FDH* and *LCR*, two genes essential for preventing the abnormal fusion process. Utilising Denaturing HPLC (Transgenomic WAVE® System), for the segregation analysis and the preparative isolation of transposon flanking sequences, we have recently cloned two more genes acting in the same pathway. Experiments to study their expression patterns and establish their functions are in progress. Using DHPLC allele segregation analysis, we were able to show that the *fdh* mutant might be complemented by *AFI*, its functional ortholog from *Antirrhinum majus* - but not with *FAE1* - a seed specific fatty acid elongase of Arabidopsis.

3-37 Extragenic suppressors of the *scarecrow-2* mutant

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Differences in cell fate are often established through asymmetric cell divisions, divisions where daughter cells acquire different identities. In plants, where cells cannot move relative to one another, asymmetric cell divisions are fundamental in setting up the multiple tissue layers and organs. In the root tip of Arabidopsis, two successive asymmetric divisions generate the endodermis and cortex cell layers of the ground tissue. First, the cortex-endodermis initial divides anticlinally producing a new initial and a cortex daughter cell. The cortex daughter cell then divides periclinally forming an endodermis cell and a cortex cell. Mutations in the putative transcription factor *SCARECROW* (*SCR*) disrupt the asymmetric division of the cortex daughter cell resulting in a single ground tissue layer of mixed endodermal and cortex identity (Di Laurenzio et al., 1996). To find new factors involved in asymmetric cell division and radial patterning of the Arabidopsis root, we screened for suppressors of the weak allele *scr-2*. Here we describe the phenotypic analysis and mapping of two recessive and six dominant *scr-2* suppressors which restore the asymmetric periclinal division of the ground tissue.

3-38 *DAL* genes and their role in plastid differentiation

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DAG (Differentiation And Greening), a gene originally isolated from a pigmentation-defective mutant in Antirrhinum majus, is required for the expression of plastid-encoded genes, very early during plastid development.
DAL (Dag-Like) is a gene family unique to plants, whose products show a transit peptide and a highly conserved N-terminal region. We have isolated insertion mutants for *AtDAL0* (the orthologue to *DAG*), *AtDAL2* and *AtDAL5* but have been unable to identify homozygous lines. In order to prove the lethality, we have crossed At*DAL0*/At*dal0* plants with plants carrying a dominant visible marker (gai phenotype) linked to At*DAL0*. We are proceeding with the rescue of the homozygous mutants. We have transformed the heterozygous plants with a construct containing each gene cloned after the promoter of *ABI3*, which allows expression just during embryogenesis. A second approach to rescue the dal phenotype would be transforming heterozygous plants with constructs that include the gene of interest between lox sites in a Cre/lox system.
We will also analyse the expression levels of the plastome genes in the mutants as compared to the wild type. We have amplified by PCR all the 87 proteins, 4 rRNAs and 37 tRNAs from the plastid genome in Arabidopsis. These PCR products are being spotted onto filters, providing a tool to assess the effect of *DAL* genes on plastome transcription. Nuclear genes which are crucial in plastid biogenesis or which are expressed in response to plastid-nucleus signal are also being spotted on the membranes.

3-39 The *DAG1* and *DAG2* DOF zinc finger genes influence with opposite effects the phytochrome–mediated pathway for seed germination in Arabidopsis

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The Dof proteins are a family of plant transcription factors ubiquitously present only in plants. These proteins are characterised by a strikingly conserved (Dof) 52 aa domain containing a single zinc finger and a downstream basic region. All Dof proteins bind similar DNA target sequences with a CTTT core. In Arabidopsis, data from the complete genomic sequence indicate the presence of 36 members of this family. We demonstrated that the Dof genes *DAG1* and *DAG2* are both involved, but with opposite roles, in the control of Arabidopsis seed dormancy and germination. In particular, inactivation of *DAG1* considerably increases the germination capability of the seeds, while mutation of *DAG2* results in seeds with a substantially lower germination potential than the wild type. All the physical and hormonal stimuli known to promote germination are almost superflous for *dag1* seeds, whereas for *dag2* seeds they are more essential than for the WT. The DAG1 and DAG2 proteins share an identical Dof domain and a high degree (77%) of aminoacid identity outside the domain. Furthermore, the *DAG1* and *DAG2* genes have identical expression patterns, limited to the vascular system of the mother plant but not of the embryo. This is in good agreement with the maternal effect of both the *dag1* and the *dag2* mutations. In addition, *DAG1* is epistatic over *DAG1*. These evidences allow to formulate a model whereby the zinc finger proteins DAG1 and DAG2 act on a maternal switch that controls seed germination, possibly by regulating the same gene(s).

3-40 Positively marked clones (PMC) to study SCARECROW function

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The ability to create mosaic animals and plants allows the phenotypic analysis of patches of genetically different cells that develop in a mutant or wild-type environment. Marked clones have been used extensively to answer developmental questions in both *Drosophila* and *C.elegans*.
We have developed a simple and efficient CRE/lox-based clonal activation system to generate and mark clones in planta either by random or directed site-specific recombination. Randomly generated clones are obtained upon heat shock (HS) treatment of plants containing the CRE recombinase driven by a HS-promoter. Generating directed clones involves CRE recombinase expression in a tissue specific manner, which is achieved using the Gal4/UAS or LhG4/pOp system. This method allows the induction of clones only in a tissue of interest. Both methods generate positively marked clones of cells that express GFP.
Whether the function of a gene is required in a given cell type is, in animal systems, often determined through the analysis of clones homozygous for a mutant allele of the gene. Similarly, the PMC system can be used to generate and uniquely label clones homozygous for a mutation of interest within an otherwise phenotypically wild-type plant.
We will describe the PMC system and analyse SCARECROW gain and loss of function clones in a mutant and wildtype background, respectively.

3-41 Genetic analysis of shoot branching in *Arabidopsis thaliana*

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Postembryonic development in higher plants depends on the activity of meristematic cell groups at the tips of shoots and roots. The aerial parts of plants are formed by the shoot apical meristem (SAM) and by lateral shoot meristems (LSMs). The SAM is established during embryonic development whereas LSMs are initiated in leave axils during postembryonic development. Considerable progress has been made in the understanding of SAM establishment and function, however, the origin of the meristematic cells forming LSMs remains unclear. In Arabidopsis, several mutants showing branching defects are known. Mutations in the *revoluta* gene lead to a reduction of sideshoot formation whereas *auxin resistant1*(*axr1-12*), *supershoot/bushy* and *max* mutants show an enhanced outgrowth of sideshoots. In addition, these mutants show other pleiotropic defects. The Arabidopsis *lateral suppressor* (*Atls*) mutant fails to initiate lateral meristems during the vegetative growth phase, however, in the axils of cauline leaves normal sideshoots are formed. The AtLs Protein belongs to the GRAS family of putative transcription factors. Double mutant analysis demonstrates that with respect to sideshoot formation the *atls-4* mutation behaves epistatic to *axr1-12*. In double mutants sideshoot formation is blocked during the vegetative growth phase. In a second approach the *atls-4* mutant was used to identify new regulators of LSM initiation. Homozygous *atls-4* seeds were mutagenized by EMS and several mutants showing an enhancement of the *atls-4* phenotype have been isolated. These mutants show a reduction of sideshoot formation in the axils of cauline leaves and are presently being characterized.

3-42 Regulation of vascular bundle patterning in the stem of Arabidopsis

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The structure of vascular bundles within the stem of Arabidopsis follows a highly ordered pattern that is laid down early in development in the differentiating inflorescence stem close to the apical meristem. The plant hormone auxin has been shown to play an important role in the initialization of vascular strands. In addition developing vascular strands are inhibited from connecting to pre-established bundles that are transporting high levels of auxin. Based on this evidence ‘lateral inhibition’ is suggested as the mechanism that gives rise to the ordered pattern of vascular strands within the stem. Interestingly, a similar mechanism involving induction and inhibition by auxin has recently been proposed to control the spacing of developing primordia within the apical meristem. The molecular regulation and signals that are responsible for this ‘lateral inhibition’ are yet to be fully understood. We have identified two alleles of a recessive mutant *continuous vascular ring* (*cov*) that have lost the ordered patterning of vascular bundles in the stem. The *cov* mutants exhibit a continuous ring of differentiated vascular tissue around the pith with little, or no, interfascicular tissue. We have isolated the gene using a map based approach. *COV* encodes a gene of unknown function. It is predicted to be an integral membrane protein and highly conserved since it exhibits homology to membrane bound proteins from several bacterial species. Analysis of how *COV* contributes to the control of vascular development and its role in the regulation of vascular patterning by auxin, are currently underway.

3-43 The Arabidopsis *twisted seed* (*tws*) mutant is affected during seed development

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Visual screening of seeds from the Arabidopsis T-DNA collection of Versailles has allowed the selection of mutants producing abnormal seeds that are potentially affected during development and/or maturation. Eighty lines out 20,000 T3 progenies screened have been selected for further study. One of these mutants, *tws* (twisted seed), presents a spiral seed phenotype. Plants homozygous for the mutation show a dwarf phenotype. Genetic analysis of the mutation demonstrated that the phenotype is linked to a T-DNA insertion. Cloning of the Flanking Sequence Tag (FST) revealed that the T-DNA is inserted in an open reading frame sharing no homology with known proteins.

Biochemical analysis of mature seeds propagated under controlled conditions was carried out. Preliminary results show that although the mutant seeds are heavier than wild type, it contains less total fatty acids. Carbohydrate content and protein pattern are also altered in *tws* seeds.

Detailed molecular characterization and cytological analyses are currently under progress on this mutant and will be presented.

3-44 Identification of *Arabidopsis thaliana* mutants altered in root architecture

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Root architecture, determines plants anchorage capacity, the efficiency of plants to exploit soil resources and the ammount of secreted compounds released by roots. Therefore, it is a relevant characteristic with important agrononomic and phytopharmacy industry implications. In our laboratory, we have screened 25000 *Arabidopsis thaliana* EMS mutagenized lines from diferent collections in order to identify mutants that have alterations in root development. Plants grown in vertical plates supplemented with MS medium with low nitrate content, we searched particularly for mutants altered in root apical dominance and spatial arrangement of lateral roots. In the first round, we selected 211 individual mutants out of which 70 were confirmed in their progeny. These mutants can be grouped in one of the following phenotypes: a) roots altered in lateral root growth angle, b) plants which lacks apical root dominance, c) plants with short root, d) roots alterated in cell wall shape and e) roots affected in obstacle avoidance response. Currently we are on the way of cloning the genes corresponding to four of these mutants by chromosome walking. In this work we will present the mutant phenotypes and their map location.

3-45 Shoot apical meristem formation: A gibberellin/auxin connection

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The *SHOOT MERISTEMLESS1* mutant (*stm-1*) displays low-penetrance alteration of lateral root emergence and defects in root epidermis cell shape. This phenotype may be partly explained by the lack of a shoot apical meristem, considered a putative source of auxin. To monitor auxin distribution in *stm-1* mutant, plants heterozygous for the *stm-1* mutation, were crossed with both *DR5* transgenic lines (in which the expression of the *GUS* reporter gene is driven by an auxin-responsive synthetic promoter) and *pNAC1::GUS* (where *NAC1* is a gene which expression is regulated by auxin). These analysis showed that *stm-1* mutants have an altered distribution of auxin that accumulates in cotyledons and shoot apex (where the meristem was supposed to form) and often disappears from the root meristems.
To investigate whether auxin mislocation in *stm-1* mutant is the cause or the effect of the shoot meristemless phenotype, transgenic lines, in which *STM* was constitutively/transiently overexpressed or silenced, were produced and analyzed. Ectopic expression of *STM* was shown to affect lateral cotyledon expansion, leaf shape, transition to flowering and flower organ formation. On the other hand, post-embryonic silencing of *STM* resulted in bushy plants with a prolonged vegetative phase and stunted inflorescence stems unable to form any flower organ. At molecular level, transient induction of *STM* was found to repress gibberellin-oxidase genes. Additional analysis suggested that alteration of gibberellin levels may be responsible for the auxin mislocation observed in *stm-1*, suggesting a close crosstalk between the two pathways in shoot apical meristem formation.

3-46 The role of *ASYMMETRIC LEAVES1* and *ASYMMETRIC LEAVES2* in Arabidopsis leaf development

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The leaves develop from the shoot apical meristem and generally exhibit bilaterally symmetrical and flattened architecture. This architecture and vascular pattern of leaves of *asymmetric leaves1* (*as1*) and *asymmetric leaves2* (*as2*) mutants in Arabidopsis are defective. Both *AS1* and *AS2* genes are required for the repression of *in vitro* shoot regeneration from and ectopic expression of class1-*KNOX* in developed leaves. AS2 protein belongs to a novel plant specific AS2/LOB family. We generated 35S::AS1, 35S::AS2 and 35S::AS2-GR transgenic plants to further define the role of AS1 and AS2 for leaf development. The accumulation of *KNAT1* transcript was reduced in 35S::AS1 but not in dexamethazone (Dex)-treated 35S::AS2-GR transgenic plants. These results indicate that AS1 is not only required but also sufficient for the repression of *KNAT1*. 35S::AS2 and Dex-treated 35S::AS2-GR transgenic plants exhibited up-curled leaves achieved via the inhibition of proliferation on adaxial surface. From these results and expression pattern of AS2, we will discuss the role of AS1 and AS2.

3-47 Active gene expression of a xyloglucan endotransglycosidase/ hydrolase gene, *XTH9*, in inflorescence apices is related to cell elongation in *Arabidopsis thaliana*

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Control of cell wall structure plays an important role in growth and differentiation in plants. Xyloglucan endotransglycosidase/hydrolases (XTHs) that catalyze cleavage and molecular grafting of xyloglucan chains function in cell wall modification. We have characterized *XTH9*, a member of the XTH family that was isolated by systematic differential screening for highly expressed genes in shoot apices in Arabidopsis. In vegetative phase, the transcripts of *XTH9* are accumulated in shoot apex region. In reproductive phase, the transcript levels in shoot apices are further increased and transcripts were also detected in flower stalks and in internodes bearing flowers. The expression levels of *XTH9* were remarkably reduced in the mutants such as the *acl* and *tfl1* mutants, in which internodal cell lengths are reduced, while the *XTH9* expression levels were recovered at permissive temperature for the temperature-sensitive *acl* phenotypes. Differential expression of *XTH9* along inflorescence stem was also detected in *pin1* where no lateral organs are formed. These observations suggest that *XTH9* expression is coordinated with plant development including differentiation from vegetative and reproductive meristems and cell elongation of inflorescence stem.

3-48 Expression analysis of *FILAMENTOUS FLOWER* using GFP in Arabidopsis

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FILAMENTOUS FLOWER (FIL) gene encodes a protein with a zinc finger and an HMG-related domains. *In situ* RNA hybridization analysis revealed that *FIL* gene is specifically expressed in the abaxial regions of cotyledons, leaves and floral organs at the juvenile stages. The adaxial epidermal cells of rosette and cauline leaves of 35S:: *FIL* plants partially changed into the abaxial epidermal cells. *FIL* gene determines the abaxial identity of cotyledons. leaves and floral organs.(*Sawa et al. Genes & Development*, 13, 1079~1088, 1999. *Siegfried, R,K, et al. Development,* 126:4117-4128, 1999).

In order to find out the *cis*-elements required for the abaxial-specific *FIL* expression, we produced a series of transgenic plants with GFP driven by truncated *FIL* promoter. Transgenic plants showed GFP expression at both the abaxial and the adaxial sides of young leaves, under the *FIL* promoter with a deletion or point mutations in a " 18bp " region. The results indicate abaxialization of *FIL* expression may be controlled by two discrete *cis*-elements within the region of the *FIL* promoter. One element is responsible for *FIL* expression at both the abaxial and the adaxial sides. Another element is due to prevention of *FIL* expression at the adaxial side.

Second, we mutagenized the seeds of *FIL* promoter::GFP plants and isolated two types of mutants, which indicate aberrant pattern of GFP signals. One type expresses very weak GFP signals. The other type showed misexpression pattern, possibly defective in the process of adaxial-specific expression.

3-49 Auxin involved in the bilateral pattern formation during embryogenesis

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In dicotyledonous plants, the apical region of the embryo acquires the bilateral symmetry in addition to the previous radial symmetry as the two cotyledon primordia develop at the opposite position around the shoot meristem. Mutations in the *PIN1* (*PIN-FORMED1*), encodes an expected auxin efflux carrier, and *PID* (*PINOID*), encodes a protein-serine/threonine kinase suggtested to enhance polar auxin transport, cause comparable defects in seedlings, as cotyledon fusion and modified number of cotyledon. The *pin1 pid* double mutantion severely suppressed the cotyledon primordia development, enhancing the phenotype of each single mutant. In these single and double mutants, we analyzed the expression pattern of three marker genes of *FIL* (*FILAMENTOUS FLOWER*), *CUC1* (*CUP-SHAPED COTYLEDON1*) and *CUC2*, the first one expressed only on the abaxial side of cotyledon primordia and the latter two expressed on the apical medial zone of the embryo. The results suggest that *PIN1* is involved in establishment of bilateral pattern and that *PID* is involved in enhancing the establishment of bilateral symmetry and that *PIN1* and *PID* coordinately promote cotyledon development through the regulation of *CUC* genes expression. In order to understand the auxin distribution in the embryo, we also analyzed the expression pattern of *YUCCA*, which encodes the enzyme involved in Trp-dependent IAA biosynthesis. Depending on these results, we want to discuss that auxin distribution through *PIN1* and *PID* is involved in cotyledon primordia development and cotyledon separation with bilateral symmetry.

3-50 Functional complementation assays for plasmodesmal trafficking: The KNOTTED1 C-terminal region contains an intercellular trafficking signal

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Previous studies showed that dominant mutations in the maize homeobox gene *knotted1* act non-autonomously during maize leaf development, suggesting that *Kn1* is involved in the generation or transmission of a developmental signal that passes from the inner layers of the leaf to epidermal cells. Follow up studies using in situ hybridization/immunolocalization and microinjection suggested that the KN1 protein itself could be the non-autonomous signal. We have shown that GFP tagged KN1 is able to traffic in the leaf and shoot apical meristem (SAM) of Arabidopsis. Here, we present a novel trafficking assay system using trichome rescue in the *glabrous1* (*gl1*) mutant. We show that a fusion of GL1 to KN1, expressed under the control of a phloem or a mesophyll specific promoter (pSUC2 or pAtRbcS2b), is able to traffic to the epidermis and rescue trichome formation in *gl1* mutant seedlings. As a negative control, GL1 or GFP~GL1 expression using the same promoters did not show trichome rescue. Furthermore we found that the C-terminal region (104 amino acids) of KN1, including the homeodomain, was sufficient to rescue trichome production when fused to GL1. To test whether trafficking might be a part of the normal function of KN1 in the shoot meristem, we also expressed the GFP~KN1 fusion protein in specific layers (L1: pAtML1 or L3: pWUS) of the Arabidopsis meristem. When expressed in L1 or L3, GFP-KN1 was able to traffic into other layers and to partially complement the strong *shootmeristemless-11* (*stm-11*) mutant allele. These trafficking and complementation results suggest that intercellular trafficking does not interfere with normal cellular function, at least in the case of GL1-KN1 or GFP-KN1. The intercellular trafficking of homeodomain proteins may therefore be a part of their normal developmental regulation. The trafficking/complementation system using a visual assay for trichome rescue provides an excellent tool for screening for trafficking defective mutants.

3-51 *HALTED ROOT* (*HLR*) gene encoding 26S proteasome subunit 4 is essential for maintenance of root apical meristem

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Root apical meristems (RAMs) containing quiescent center (QC) control root growth through balanced cell division and differentiation. Although normal root growth requires maintenance of RAM activity, little is known about this maintenance machinery.

We isolated an Arabidopsis mutant, *halted root* (*hlr*), which exhibits aberrant post-embryonic root growth. We analyzed changes of RAM activity throughout root growth using various marker genes. In *hlr*, normal RAM structure established during embryogenesis was disturbed with disordered cell division plane within 36 hours. At this time, a QC-specific marker missed its expression in *hlr*. In addition, another QC-specific marker expressed 3 days after germination in wild-type root was not detected in the mutant. These results suggest that *hlr* cannot establish QC activity and fails to maintain normal pattern of cell division immediately after germination.

We have cloned the *HLR* gene and found it encodes a homologue of proteasome subunit 4. Proteasome is a huge complex that degrades poly-ubiquitinated proteins. Arabidopsis has another one gene encoding 99% identical protein to HLR, so that we named this gene *HLR-LIKE PROTEIN* (*HLP*). RNA gel blot analysis revealed that both of *HLR* and *HLP* genes are expressed in all organs. We isolated *HLP* knockout plant (*hlp* mutant) from T-DNA inserted lines. Although *hlp* did not show any morphological abnormality including disrupted RAM structure, *hlr hlp* double mutant was gametophytic lethal. These results indicate that both genes are required for normal development and that *HLR*, rather than *HLP*, has major role in RAM maintenance.

3-52 *short stem and midrib*, an Arabidopsis mutant defective in stem and midrib elongation

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short stem and midrib (*ssm*) is a novel semi-dwarf mutant whose height at maturity reaches only a half of that of the wild-type Columbia strain as a result of shortened internodes. While root and hypocotyl lengths of the etiolated and light-grown mutant seedlings are normal, rosette leaves are shorter in *ssm* than in the wild type and ssm leaf blades are severely waved because of specific defects in midrib elongation. Sections through inflorescences in *ssm* revealed that the shape of the inflorescence meristem and cell files in stem internodes are similar to the wild type but cell lengths in each cell file are reduced. Although the *ssm* mutant shows responses to exogenous application of GA in a manner similar to the wild type, it can not restore the *ssm* phenotype.

The *ssm* mutant was derived from a T-DNA transformed population, but it has been shown that the *ssm* locus is not tagged by a T-DNA. Furthermore, we found that, while the *ssm* mutation behaves as a single recessive locus in the Columbia background, appearance of the *ssm* mutant phenotype requires another recessive mutation in the Landsberg background, which is harbored by the wild-type Columbia strain. Progress of the positional cloning of these two *ssm* loci will be presented.

3-53 Auxin mediated cell cycle regulation during early lateral root initiation

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Despite the considerable amount of research that has been done, the exact regulatory pathways leading to lateral root initiation are still poorly understood. The difficulties may have stemmed from the apparent spatial and temporal asynchrony of the initiation events. Here, we present a novel synchronized lateral root inducible system in Arabidopsis that allows detailed molecular analysis of the early lateral root initiation. This system is based on seed germination in the presence of the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) and early transfer to the exogenous auxin 1-alpha-naphthalene acetic acid (NAA), to prevent and induce pericycle activation, respectively. In this system the whole pericycle was activated for lateral root initiation in an enhanced manner. The auxin penetration in the roots caused fast down regulation of two CDK inhibitor genes (*KRP1* and *KRP2*) followed by synchronous cell cycle reactivation from the G1 gap phase. *KRP2* was shown being targeted for auxin-mediated regulation and its over-production strongly affected pericycle activation. To further elucidate the molecular regulation during lateral root initiation we are currently performing genome-wide expression analysis on the system using cDNA microarray and cDNA-AFLP techniques. Our results show that the system will allow us to identify and sort regulatory genes involved in the early processes of root branching in plants. For example, on the microarray experiments we have been able to follow the induction of cell cycle regulatory genes sequentially with repression of lateral root suppressor *IAA28* and induction of early and late auxin responsive genes such as *IAA2* and *IAA7*.

3-54 Functional analysis of the barley CslC gene family

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Cell walls are central to the growth and development of cereals, and are important contributors to the overall quality ofcereal grains. Variation in the fine structures of major wall polysaccharides can effect the functional/ rheological properties of cereal-based products. The genes and enzymes that control cell wall biosynthesis are largely unknown. The recent cloning of a plant cellulose synthase gene (Arioli *et al.,* 1998; Pear *et al.,* 1996) and the identification of mutants in cell wall components (Peng *et al.,* 2000; Taylor *et al.,* 1999; Arioli *et al.,* 1998) has however, paved the way for genetic tailoring of wall phenotypes in grasses. We are using barley as a model system to study cell wall synthesis in grasses. Of particular interest is wall regeneration by protoplasts and wall elongation in coleoptiles. We have focussed our efforts on the CslC family as a likely source of genes encoding polysaccharide synthases of matrix phase wall compounds.
To date four CslC genes have been identified and mapped in barley. Expression analysis has been monitored by Northern blots as well as RT-PCR and *in situ* PCR. Recombinant bacterial proteins are being used for antibody production to determine the cellular localization of CslC gene products. To determine the function of these genes we have developed a system that allow us to transiently silence genes in barley immature embryos and coleoptiles.

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3-55 The *BLADE-ON PETIOLE* gene is required for maintenance of the determinate state of organs in Arabidopsis

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In higher plants, leaves are initiated in succession around the periphery of the shoot apical meristem (SAM). The mechanism by which the indeterminate SAM gives rise to determinate leaf organs is one of the key questions in understanding plant development and pattern formation. We identified an Arabidopsis mutant in which the meristematic activity in leaves is uncontrolled. As a recessive mutant allele, the *blade-on petiole* (*bop*)*1-1* mutant results in ectopic, lobed blades along leaf petioles. Histological analysis revealed that these ectopic organs were caused by ectopic meristematic activity in the basal parts of petioles. Furthermore, three class I *knox* genes, namely, *KNAT1*, *KNAT2* and *KNAT6*, were misexpressed in *bop1-1* leaves. By contrast, expression of the *SHOOTMERISTEMLESS* (*STM*) gene, which is also a class I knox gene was suppressed in the SAM region. The *bop1-1* mutation acted synergistically with mutations that are related to meristem functions, namely, *as1*, *as2* and *stm-1*, suggesting that it plays a redundant and novel role in the regulation of meristem function. We propose that the *BOP1* gene might function to repress expression of class I *knox* genes and its function is very important for the leaf morphogenesis. Cloning of *BOP* gene is now underway.

3-56 Effects on plant development by mutation of plastid protein import apparatus

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Plastids perform a number of essential biochemical functions such as photosynthesis, fatty acid biosynthesis, amino acid biosynthesis, and sulfate and nitrate reduction. Since most of the proteins associated with these biochemical reactions are encoded in nucleus, they are synthesized as precursor proteins and are imported into plastids 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 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 remains to be proven *in vivo*. Arabidopsis plants that have T-DNA insertions into the putative homologs for the chloroplast protein import machinery have been isolated. The morphological and biochemical data demonstrate that the *in vivo* role of the translocon component during plant development.

3-57 Expression analyses of the HD-GL2 gene family in *Arabidopsis thaliana*

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The subclass of plant-specific homeodomain-leucine zipper (HD-ZIP) transcription factors, named HD-GLABRA2 (HD-GL2), include GL2, FWA, ANTHOCYANINLESS2 (ANL2), MERISTEM LAYER1 (ATML1) and PROTODERMAL FACTOR2 (PDF2). Based on their mutant phenotypes and gene expression patterns, some members of the *HD-GL2* gene family have been implicated in the transcriptional regulation of epidermal layer-specific gene expression. The recombinant ATML1 and PDF2 gene products can bind to the L1 box *in vitro*, which has been identified as a cis-regulatory element required for L1-layer-specific gene expression. Promoter regions of *ATML1* and *PDF2* also contain the L1 box, suggesting that these transcription factors form an autoregulatory loop for their expression. We made transgenic plants carrying the *PDF2* promoter-GUS fusion construct and confirmed that it conferred L1-specific expression of the GUS activity. Disruption of the L1 box in the construct eliminated the GUS expression.
To define further the function of the *HD-GL2* gene family, we extended our analysis to other uncharacterized members belonging to the family. Database search revealed that the Arabidopsis genome contains 16 genes of this family. Expression patterns of these genes and analysis of some of the knockout mutants will be presented.

3-58 Is trehalose-6-phosphate required in development because it controls primary metabolism?

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Trehalose metabolism is ancient and found in most organisms, plants included. Trehalose accumulated in quantitative amounts is a stress protectant. Plants generally contain only trace amounts of this sugar however, too little for protection, and the question on the role of trehalose metabolism in plants remains.
Transgenic Arabidopsis over-expressing *E.coli* trehalose-6-phosphate synthase (TPS), phosphatase (TPP) and hydrolase (TPH) or trehalase are characterised.
TPP and TPH over-expressing Arabidopsis seedlings are sensitive to sugar supplied in the medium. They contain low levels of trehalose-6-phosphate (T6P) and increased levels of phosphorylated hexoses, which further increase when seedlings are grown on glucose, fructose or sucrose. This resembles yeast deleted in TPS and establishes T6P as a regulator or signal in primary metabolism of plants.
Arabidopsis lacking AtTPS1 are embryo lethal with embryos developing slower than wt and arresting at the seed filling stage when sucrose levels in seeds rise to high levels. Eastmond et al. 2002 (Plant J. 29(2):225-35) have previously shown that embryos lacking AtTPS1 are sensitive to sucrose in culture. Our data suggests that T6P is required to rescue AtTPS1 deleted embryos *in planta.* Like in seedlings, T6P might therefore be necessary for sucrose assimilation and prevent hexose phosphate accumulation in embryos.
We are developing approaches to identify intermediates and targets in T6P mediated regulation of primary metabolism. This regulation could be the basis for the altered vegetative and embryonic development observed when tampering with trehalose metabolism.

3-59 Sterols integrate auxin and ethylene signalling in Arabidopsis morphogenesis

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The *hydra* mutants of Arabidopsis have a pleiotropic phenotype causing severe pattering and morphological defects in the embryo and seedling (Topping et al 1997: Development 124, 4415-4424). The *HYDRA1* gene encodes a D8-D7 sterol isomerase, and the *HYDRA2* gene encodes a sterol C-14 reductase, allelic with the *FACKEL* gene product (Souter et al. 2002: Plant Cell in press). Mutations in the *HYDRA* genes result in dwarfed, seedling lethal plants with stunted roots and prolific root hair patterning. The mutant phenotype is associated with gross changes in the sterol profile, and cannot be rescued by exogenous application of plant sterols or brassinosteroids. Patterning defects are evident in the *hydra* mutants from the early globular stage of embryogenesis, and constructs of the b-glucuronidase reporter fused to the sterol isomerase promoter region show constitutive expression throughout the globular and heart stages, as well as strong expression throughout root development. Auxin and ethylene signalling is defective in these mutants. The auxin-regulated DR5 and ACS1::GUS reporter constructs showed mis-expression in *hydra* seedlings, which have an increased sensitivity and/or cell permeability to auxin. Inhibition of auxin and ethyene signalling in the *hydra* mutants leads to rescue of many aspects of the mutations. These results suggest a role for plant sterols in the integration of auxin and ethylene signalling in Arabidopsis.

3-60 Regulation of plant organogenesis by auxin

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Leaf and flower formation take place at the periphery of the shoot apical meristem. Organ formation depends on polar auxin transport (PAT). In Arabidopsis plants flower formation can be inhibited either chemically by inhibiting PAT with 1-N-naphthylphthalamic acid (NPA) or genetically by mutations in the auxin efflux carrier *Pin1*. Meristem self-perpetuation and the formation of stem tissues are not affected (Vernoux et al., 2000), resulting in the formation of continuously growing stalks, devoid of lateral organs (pins). Flower formation can be restored by the local application of IAA to the periphery of the pin meristem (Reinhardt et al., 2000). In contrast to effects obtained with a non-functional auxin efflux carrier, mutations in the auxin influx carrier *Aux1* do not result in a cessation of flower formation. In tomato plants however, chemical inhibition of the auxin influx carrier with naphthoxy-1-acetic acid (NOA) interferes with leaf positioning at the shoot apex, suggesting a central role for the auxin influx carrier in organ positioning. The phenotype of Arabidopsis pin1/aux1 double mutants and flower formation and positioning after application of auxin were analysed. The function of auxin in the regulation of plant organogenesis is presented in a model.

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Vernoux, T., Kronenberger, J., Grandjean, O., Laufs, P. and Traas, J. (2000) PIN-FORMED 1 regulates cell fate at the periphery of the shoot apical meristem. Development 127, 5157-5165.

3-61 Functional analysis of Arabidopsis L1 layer-specific homeobox genes

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The shoot apex of angiosperms consists of clonally different cell layers from the outside to the inside and the 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. The *Arabidopsis thaliana PROTODERMAL FACTOR2* (*PDF2*) and *MERISTEM LAYER1* (*ATML1*) genes encoding HD-GL2 class proteins show 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. Here we report the characterization of T-DNA insertion mutants of both *PDF2* and *ATML1*. Our results suggest that PDF2 and ATML1 are functionally interchangeable and play a critical role in establishing and maintaining the L1 cell layer possibly by regulating the expression of L1-specific genes.

3-62 The anatomical basis of the leaf phenotype of the *reticulata* mutant

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In leaves of the reticulata mutant of Arabidopsis,the vascular network can be clearly distinguished as a green reticulate pattern on a paler lamina. In two alleles of *reticulata* the underlying basis for the phenotype is a marked reduction in the density of chloroplast-containing cells per unit leaf area in the interveinal regions, with a concomitant increase in airspace. Additionally, the density of the vascular network in first leaves of *reticulata* is reduced relative to wild type. This suggests that the *reticulata* mutation specifically perturbs ground cell division early in leaf primordia development, with reduced cell proliferation affecting the vascular network as well as mesophyll cell number. In spite of the altered internal architecture, *reticulata* leaves are of normal shape, suggesting that the epidermis plays a major role in regulating leaf shape in Arabidopsis, whereas the correct development of the mesophyll tissue is more important in the control of leaf thickness.

3-63 *DORNROESCHEN (DRN)* a novel function in the shoot apical meristem of *Arabidopsis thaliana* affects stem cell fate

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A gain-of-function mutation was identified in an ERF-type (Ethylene Response Factor) transcription factor in an activation tagging approach. According to an arrest of shoot meristem activity and thornlike appendages in the dominant *drnD*allele the gene was named *DORNROESCHEN (DRN)*. In wildtype *DRN* transcripts are detected in the 4-cell embryo and later restricted to the apical layers of the embryonic SAM. During vegetative, inflorescence and floral development DRN activity is constitutively found at the tip of the SAM and transiently detected in the anlagen of most lateral organ primordia. Ectopic expression of *DRN* in the dominant *drnD* allele causes dramatic changes in the organisation of the SAM and intermingling of the *STM*, *CLV3*, and *WUS* patterns, which may explain the arrest in SAM activity. A double mutant analysis indicates that the consequences of ectopic *DRN* activity in the *drnD* allele do not dependent on the *WUS, CLV* or *STM* functions. Due to the absence of an obvious phenotype a *drn* loss-of-function allele would have escaped detection in classic mutagenesis screens.

3-64 Auxin synthesis in embryos

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In vitro manipulation of zygotic embryos with auxins, antiauxins and auxin polar transport inhibitors led to a wide range of morphogenetic alterations indicating that auxin and its spatial distribution play an essential role in embryonic pattern formation.
In a recent study, we showed that the distribution of [3H],5-N3IAA, an analog of indole-3-acetic acid (IAA), was heterogeneous and changed during embryo development. In particular, the shift from radial to bilateral symmetry was correlated with a redistribution of [3H],5-N3IAA in the embryo that may be achieved by active polar transport to specific embryonic regions. In order to gain further information on underlying mechanisms by which auxin acts to regulate embryonic pattern formation, attention was devoted to determine the origin of the IAA pool(s) present in the embryo. We investigated the spatial and temporal dynamic of IAA synthesis during embryo development of Arabidopsis and wheat, chosen as model for dicot and monocot respectively. Multiple pathways for IAA *de novo* synthesis exist in plants depending on the developmental stage, the tissue and the plant. In the last step of the indole-3-acetonitrile (IAN) pathway, IAN is converted to IAA by nitrilases. An immunocytochemistry approach was used to visualize the expression patterns of nitrilase protein(s) during seed development.

3-65 Overriding the influence of guard mother cell shape on selection of the orientation of the division plane

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More than 100 years ago, biologists recognized that knowing the cell shape or its direction of expansion, it is possible to predict the orientation of its future cell division plane. In 1863, Hofmeister pointed out that cells divide in a plane perpendicular to the main growth axis (1). Twenty-five years later, Errera noted that cell division takes place so that the cell division plane has the minimal area for cutting the volume of the cell in half (2). Direct evidences supporting these rules come from elegant cell deformation experiments in which cells divided, following the Hofmeister and Errera' rules, in a plane parallel to the compressive force (3,4). Here we show that, unexpectedly, the stomatal precursor (the guard mother cell, GMC) does not follow these general rules: it divides parallel to its long axis. In addition, this strict orientation of the GMC division plane seems not to be essential for stomata formation.

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3-66 Characterization of *ZmOCL* homeo box genes and identification of putative interacting proteins

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The formation of a morphologically distinct outer cell layer or protoderm is one of the first and probably one of the most important steps in patterning of the plant embryo. A family of homeo box genes (*ZmOCL1*, *ZmOCL3, ZmOCL4* and *ZmOCL5*) has been described previously and showed in maize essentially L1 or epidermis-specific expression. However, each gene is expressed in a disinct region of the embryonic L1 cell layer during early development. These genes encode proteins of the plant specific homeodomain-leucine zipper (HD-Zip) transcription factors family. The presence of a putative leucine zipper domain immediately downstream of the homeo domain in OCL proteins, in combination with their overlapping RNA expression patterns, raises the possibility that they could interact in combination as homo or hetero-dimers. By using the yeast two hybrid system we demonstrated that OCL1 and OCL4 could act as transcription activators. We showed that hey are able to form homodimer and hetero dimer with other members of the familly. We have also identified differents putative interacting proteins for these two OCL proteins.

3-67 Identification of genes involved in Arabidopsis root development

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Using an enhancer detector and gene trap collection of about 1000 transposant lines of *Arabidopsis thaliana* we carried out genetic screenings looking for altered phenotypes and GUS reporter gene expression in the roots of plants growing under normal and low phosphorous conditions.
We identified a group of about 30 lines showing reporter gene expression in roots. In some cases the level of expression and tissue specificity vary in accordance with nutritional status. We also screened for mutants altered in root architecture. Sequencing of genomic DNA flanking the insertion site show the diversity of genetic products involved in root development, including genes with homology to transcription factors, hydrolytic enzymes, and structural proteins. Many of the identified lines express GUS in the epidermis and stele; however, we also found lines expressing the reporter gene in each of the root tissues.
Two lines with altered root architecture were studied in more detail. In the first one, cellular elongation is inhibited in darkness and decreased in optimal growth conditions. Root hair elongation is also decreased. Reporter gene expression is confined to the central cylinder in the absence of stress conditions, while stressed plants loose the tissue-specific expression. The protein encoded by this gene is similar to proteins involved in cytoskeleton organisation. The second line shows alterations in lateral roots distribution, which develops without following a clear pattern. This gene encodes for a cell wall hydrolytic enzyme that seems to allow the development of lateral root primordia across outer cell layers.

3-68 *TOO MANY MOUTHS* encodes a receptor-like protein that modulates epidermal patterning in Arabidopsis

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Stomata control gas exchange and water loss and are therefore key to the survival of land plants. In Arabidopsis as well as other plants, both the number and distribution of stomata are regulated during leaf development, yet the genes controlling this process are poorly understood. Stomata are formed after a series of asymmetric divisions of transiently self-renewing precursors termed meristemoids. Stomata are continually produced during the mosaic development of the leaf, and many arise through division of cells immediately adjacent to pre-existing stomata. Correct spacing of stomata results when the plane of formative asymmetric divisions is oriented so that the new precursor, the satellite meristemoid, does not contact the pre-existing stoma or precursor. Developmental evidence suggests that intercellular signaling provides spatial cues that regulate division orientation and may also block asymmetric division in cells adjacent to two stomata or precursors. Mutations in the gene *TOO MANY MOUTHS* disrupt patterning by randomizing the plane of formative asymmetric divisions and by permitting ectopic divisions in cells adjacent to stomata or precursors. We cloned *TMM* and found it encodes a leucine-rich repeat receptor-like protein that is expressed in proliferative post-protodermal cells. Our observations suggest that TMM functions in a signaling pathway that reads positional cues to control the plane of patterning divisions as well as the balance between stem cell renewal and differentiation in stomatal and epidermal development.

3-69 The pleiotropic mutation *dar1* affects meristem development in *Arabidopsis thaliana*

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Shoot architecture is shaped upon the organogenic activity of the shoot apical meristem (SAM). Such an activity relies on the balance between the maintenance of a population of undifferentiated cells in the centre of the SAM and the recruitment of organ founder cells at the periphery. A novel mutation in *Arabidopsis thaliana*, *distorted architecture1 (dar1)*, is characterised by disturbed phyllotaxy of the inflorescence and consumption of the apical meristem late in development. Those traits suggest a defect in meristem function as was confirmed by light microscopy and SEM analyses on *dar1* SAM at different stage of plant development. Light microscopy analyses revealed an abnormal cell size and less well defined cell layers in *dar1* SAM than in the wild type while irregular partitioning of meristematic domains was evident from the SEM analyses. *dar1* was also found to genetically interact with two mutants known to affect the SAM structure and function, *wushel* and *mgoun1*, substantiating the involvement of DAR1 in meristem development and suggesting primordium initiation at the stage of cell recruitment as the probable issue. Moreover the mutant showed an alteration of the root apical meristem (RAM) structure indicating that the RAM activity likely is affected in a similar fashion as the SAM organization. Taken together those observations point to a role for *DAR1* in meristem maintenance possibly playing an essential role in a context of cell division and/or differentiation.

3-70 FIE a Polycomb protein regulate reproductive and vegetative development

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A lesion in the *FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)* gene initiates endosperm and fruit development without fertilization. *FIE* encode a WD polycomb (PcG) protein. In insects and mammals, homologous proteins are known to regulate developmental programs. PcG protein complex control gene transcription via the modulation of chromatin structure. Previously we have shown that FIE interact *in-vitro* with *MEDEA (MEA*) a SET domain protein. These evidence support our hypothesizes that FIE may form a complex with other PcG proteins to control endosperm development until fertilization occurs as well as regulating embryo development after fertilization by controlling the expression of transcription factors. So far homozygous *fie* mutant adult plants were not available due to the abortion of embryos bearing the maternal *FIE* mutant allele. In this work we have used transgenic plants, in which *FIE* expression was silenced, to explore the function of FIE in adult plants. Mutant plants displayed abnormal development of both vegetative and reproductive organs as well as shortening of flowering time. These abnormalities correlated with the expression of specific target genes that were up-regulated. Characterization of FIE silenced plants will be presented and the role of FIE in the development of both the vegetative and reproductive phases will be discussed.

3-71 AtFIP37, a partner of AtFKBP12, has an important role in Arabidopsis embryo development and trichome morphogenesis, presumably by acting in pre-mRNA alternative splicing

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FKBPs (FK506 binding proteins) represent one class of immunophilins, a large family of peptidyl-prolyl-cis-trans isomerases, also named rotamases or PPiases, involved in protein folding in a wide range of organisms. They were initially described in animals as the target of the immunosuppressive drugs FK506 and rapamycin. FKBPs have been implicated in various signal transduction pathways but their function is still unclear, particularly in plants. In the model plant Arabidopsis, 17 FKBPs have been predicted. Here we report studies on AtFIP37, a protein recently identified as a two-hybrid partner of AtFKBP12. Expression analysis of AtFIP37, by way of real-time RT-PCR, western-blot and promoter-GUS fusion, show a constitutive expression pattern, suggesting a housekeeping role of that gene. Loss of AtFIP37 function in a T-DNA mutant line confirms this hypothesis as it induces embryo lethality with embryos blocked at the globular stage. YFP-fusion experiments have shown the nuclear localisation of the protein in speckles reminding splicing factor compartments (SFCs). This localisation is consistent with that of two animal pre-mRNA alternative splicing factors which display significant homology with AtFIP37. Interestingly, transgenic lines over-expressing AtFIP37 show over-developed trichomes on their rosette leaves with increased branching and ploidy, a peculiar phenotype similar to that of plants over-expressing SRp30, a serine/arginine-rich alternative splicing factor. Our findings suggest that AtFIP37, which action could be regulated by AtFKBP12, plays an important role in plant development, presumably in alternative splicing.

3-72 A huge gene to prevent huge trichomes: *KAKTUS* encodes a ubiquitin ligase related protein

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Arabidopsis leaf trichomes result from the outgrowth and branching of a single epidermal cell. The committed trichome cell endoreplicates several times before and during outgrowth, whereas neighbor cells keep dividing. Previous genetic analysis has defined 5 genes that together restrain trichome cell overgrowth, branching and endoreplication cycles: *KAKTUS, POLYCHOME, RASTAFARI,* the gibberellin repressor *SPINDLY*, and the patterning gene *TRIPTYCHON*. Here, a tagged T-DNA line with overbranched trichomes has been isolated in the Wasilevskaia ecotype. This phenotype always cosegregated with kanamycin resistance (d< 2cM). The T-DNA insertion is allelic to the EMS mutant *kaktus-3*. The insertion disrupts a coding sequence showing homology to the HECT family of E3 ubiquitin-ligases. Thanks to the TILLING project (http://tilling.fhcrc.org:9366/), an EMS mutation introducing a premature opale codon upstream of the HECT domain has been isolated: it also results in a strong *kaktus* phenotype. We propose that the *KAKTUS* gene corresponds to the merging of two successive annotations in tandem on Chromosome 4, making a 13 kb gene encoding a 1900 amino acid translation product. Attempts to rescue the *kaktus* phenotype with the wild-type gene will be reported. Within the HECT family, KAKTUS shares stronger homologies to a subset of E3-ligases including human, yeast, and other plant proteins; these homologies extend far upstream of the C-terminal catalytic end, into a domain related to alpha-karyopherins. This conserved sub-family might share conserved targets. Double mutant analysis points to transcription factors GLABRA3 and GLABRA1 as candidate targets.

3-73 Light and exogenously supplied sugars interact to modulate stomatal development in *Arabidopsis thaliana*

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Light is an important factor in the life of a plant. It provides the energy needed for CO2 conversion into biologically useful molecules such as sugars and it also constitutes a signal for proper plant responses to its environment. On the other hand, plants respond to intrinsic signals, such as internal sugar levels, that sense their physiological status. The integration of both types of signals is necessary for physiology and development. Our results show that both exogenously supplied sugars and light promote stomatal production and modulate stomatal pattern in *Arabidopsis thaliana*, and that they also act together to produce a synergistic effect. The light effect is, at least in part, mediated by specific photoreceptors, because *phyA*/*phyB*/*cry1* triple null mutants developed stomata at a significant lower rate than their wild-type controls when grown in the light without any sugar source. The role of these photoreceptors must be redundant, since *phyA*, *phyB* and *cry1* single mutants did not show any differences with respect to their wild-type controls. Only metabolizable supplied sugars increased stomatal development, suggesting that their effect may be mediated through general metabolic responses. However, the behaviour of *cop1* indicates that sugars act probably as signals and that they converge with the light-regulated pathway upstream from the COP/DET/FUS components: *cop1* mutants grown in the dark and without any sugar source develop stomata at a significant higher rate than their wild-type controls, being comparable to wild-type plants grown in the light with a sugar source. This behaviour also suggests that the stomatal development arrest experienced by wild-type plants when they grow in the dark and in the absence of added sugars is not just due to the depletion of storage products as source of energy, but to a block into the stomatal development pathway.

3-74 Identifyng downstream targets of HD-ZIP III transcription factors

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The Arabidopsis HD-ZIP III family of transcription factors is composed by five highly related proteins characterized by the presence of a putative sterol/lipid binding domain (START). Interestingly, all the corresponding genes are expressed in the vascular meristems. *ATHB-8* is an early marker of procambial cells positively regulated by auxin. Moreover, we have shown that *ATHB-8* acts as a differentiation-promoting transcription factor of the vascular meristems. *INTERFASCICULAR FIBERLESS1/REVOLUTA (IFL1/REV)* is necessary for interfascicular fibers differentiation and proper vascular tissue development as well as for lateral meristems initiation and normal organ formation (Talbert et al., 1995; Zhong & Ye, 1999; Ratcliffe et al., 2000; Otsuga et al., 2001). *PHABULOSA (PHB)/ATHB-14* and *PHAVOLUTA (PHV)/ATHB-9*, have been implicated in defining and/or interpreting positional identity along the plant's radial axis and in the establishment of polarity in the leaf (McConnell et al., 2001). Consistently with these more general effects, *PHV/ATHB-9, PHB/ATHB-14*, and *IFL1/REV*, are also expressed in apical meristems and in the adaxial domain of the developing leaf. To gain more information on gene function, we have identified several new alleles of *hd-zip III* mutants using a reverse genetic approach and we are currently combining them to generate double and higher order mutants. These genetic resources, along with transgenic 35S::ATHB-8 plants, are also being exploited to investigate expression profiles of putative target genes. In addition, in order to identify primary target genes, we have generated plants expressing the chimeric ATHB-8::GR protein to determine transcript profiles in the background of posttranslationally controlled HD-ZIP III gene activity.

3-75 Regulation of the placement of the embryonic shoot-root axis by the *TILTED* locus

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The placement of the body's axes of symmetry (shoot-root and central-peripheral in plants) 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 will present a preliminary characterization of the tilted (til) mutant of Arabidopsis. Embryos homozygous for the mutation (in a heterozygous maternal environment) show a tilting of the shoot-root axis with respect to the embryo-suspensor axis during the globular and heart stages of development, as revealed by shoot and root pole markers (SHOOTMERISTEMLESS, PIN-FORMED4, SCARECROW). The development of the mutant embryos is delayed with respect to that of their non-mutant siblings, but they recover afterwards, eventually producing normal seeds. Homozygous embryos in a homozygous maternal environment show a more severe phenotype. Homozygous mutant plants develop normally until flowering, but then they present very reduced fertility. Either the ovules of these plants are not fertilized or the embryos arrest (maternal effect). The til locus maps to the top of chromosome 1, and we are in the process of mapping it more finely. Our results suggest that the TIL locus is required maternally for megagametophyte function, and zygotically for embryonic axis placement.

3-76 Initial characterization of Pumilio homologues from Arabidopsis

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Four Pumilio-like genes were found in *Arabidopsis thaliana*, three of which are located in chromosome II (APUM-1, -2, and -3) and are very similar to each other, and the fourth is in chromosome IV (APUM-4). The APUM-2 promoter was used to drive expression of either of the reporter genes GUS or GFP in transgenic plants containing the construct. We found that both GUS and GFP were expressed in both shoot and root meristems. In the shoot apex, the APUM-2 promoter seems to drive expression in the peripheral meristem outside of the central zone of the meristem. In the root apex, reporter expression was found in the meristematic region above the collumela, and also in the cells adjacent to nascent secondary roots. These data suggest a role for APUM-2 in meristem development of both shoot and root, a very interestesting possibility since only the genes FASCIATA 1 and 2 have been found to act in both meristems of Arabidopsis.

3-77 Novel genes with the expression at various stages of vascular development in zinnia and Arabidopsis

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Vascular system contains several types of cells such as tracheary elements (TEs), sieve elements, xylem and phloem parenchyma cells, and fibers. To elucidate the molecular mechanisms underlying vascular development, we carried out comprehensive analysis for genes expressed during vascular development using the *in vitro* culture system in which mesophyll cells isolated from young zinnia leaves differentiate into TEs. An equalized cDNA library was prepared from the cultured zinnia cells that were differentiating into TEs. We prepared microarrays for more than 9,000 cDNA clones from the library and examined the expression profiles during the *in vitro* differentiation of TEs. As a result, we identified more than 200 genes that would be expressed specifically during vascular development. Next, based on these results, we searched Arabidopsis counterparts. Arabidopsis genes with higher sequence similarities to the putative zinnia vascular-specific genes were selected and their expression patterns were examined by promoter-reporter assays in Arabidopsis. For the assays, we prepared several kinds of binary vectors using the gateway cloning systems to fuse promoter regions with reporter genes (CFP, YFP, or GUS) at a time easily. To date we have already assayed the expression patterns of more than 50 Arabidopsis genes and identified at least 30 genes with the expression specific to various types of cells and various developmental stages in vascular system. These genes will be good tools to dissect the molecular bases underlying vascular development.

3-78 *clv1-1* is a dominant negative mutation

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The shoot apical meristem (SAM) is responsible for generating all above-ground organs in the plant. This function requires the balance between the maintenance of a population of dividing undifferentiated cells at the center of the meristem and the direction of appropriately positioned descendants of these undifferentiated cells toward organ formation and eventual differentiation. The *clavata1* (*clv1*) mutations disrupt this balance, leading to an alteration in the structure and function of the SAM in *Arabidopsis thaliana*. The *clv1* mutant plants exhibit defects in the inflorescence meristem, leading to enlarged SAM, club-shaped siliques and extra flower organs. Detailed analysis indicates that the function of *CLV1* is to promote the transition of undifferentiated cells toward organ formation. The *CLV1* gene encodes for a receptor belonging to the family of leucine-rich repeat receptor-like kinases (RLK). Genetic and biochemical analysis show that CLV3 is the ligand of CLV1. *clv1* alleles of varying severities exhibit phenotypes ranging from weak (*clv1-6, clv1-7*) to strong phenotypes (*clv1-4, clv1-1*). Here we provide evidence that intermediate/strong *clv1* alleles are dominant negative. First, we have isolated *clv1* null alleles that exhibit weak phenotypes. Second, co-suppression of *clv1-1* mRNA expression partially suppresses the clv1 phenotype. We propose that additional RLKs are active within the meristem that have functional overlap with CLV1 and are also activated by CLV3. These RLKs are presumably interfered with by the proteins encoded by dominant-negative *clv1* alleles.

3-79 Insight into cellular processes governing embryo development by *LEC2*

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Plant embryos form by several developmental pathways including zygotic embryogenesis, somatic embryogenesis, microspore embryogenesis and apomixis. Induction of embryo development by all of these pathways requires the establishment of an embryogenic environment, although little is known of the molecular processes governing this change in developmental fate. *LEAFY COTYLEDON2 (LEC2)* encodes a B3 domain transcription factor that serves critical roles during both the early and late phases of embryogenesis. Moreover, ectopic *LEC2* expression is sufficient to induce somatic embryogenesis in vegetative cells in the absence of hormone treatments normally required for induction of somatic embryogenesis (Stone et al. 2001 PNAS 98:11806). Only one other gene, *LEC1*, which encodes a subunit of CCAAT binding transcription factor complex is known to induce somatic embryo formation by ectopic expression (Lotan et al. 1998 Cell 93:1195).
To define the cellular processes by which *LEC2* establishes an embryogenic environment, we analyzed the postembryonic phenotype of plants expressing *LEC2* ectopically from the *35S* promoter. In addition to somatic embryogenesis, *35S::LEC2* induced a number of striking phenotypes including storage reserve accumulation in non-seed tissues, floral abnormalities including male sterility and root induction on flowers, shoot/ leaf organogenesis in the absence of exogenous hormones, and small plants with limited internode elongation and reduced apical dominance. Mechanisms underlying the effects of *LEC2* on plant development will be discussed.

3-80 The LOB-DOMAIN gene *LBD6* is important for leaf development

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The *LATERAL ORGAN BOUNDARIES* (*LOB*) gene is expressed at the base of lateral organs that initiate from the shoot apical meristem. *LOB* encodes a novel plant-specific protein of unknown function that shares a conserved domain with 42 other Arabidopsis proteins. We have named these related genes *LOB DOMAIN* (*LBD*). *LOB* expression is reduced in plants containing mutations in the *SHOOT MERISTEMLESS* (*STM*), *ASYMMETRIC LEAVES1* (*AS1*), or *AS2* genes, indicating that the activities of these genes are needed for proper *LOB* expression. Loss-of-function mutations in *LOB* do not result in an obvious phenotype, suggesting that *LOB* may be functionally redundant. We are taking a functional genomics approach to study the *LBD* gene family, and are analyzing the four *LBD* genes that are most closely related to *LOB*, *LBD6*, *LBD10*, *LBD25*, and *LBD36*, using double stranded (ds) RNA suppression and over-expression approaches. dsRNA suppression of *LBD6* results in the formation of lobed leaves. This phenotype is similar to that caused by ectopic expression of the *KNAT1*/*BP* homeobox gene, and to phenotypes resulting from loss-of-function mutations in *AS1*, *AS2* or *SERRATE* (*SE*). We have shown that the *as2* phenotype results from a mutation in the *LBD6* gene. Ectopic expression of high levels of *AS2* results in a dramatic whole-plant phenotype. *35S::LBD6* plants are small, the leaf lamina often fails to expand, and outgrowths develop on the leaf surface. *35S::AS2* plants that express moderate levels of *AS2* exhibit a bp-like phenotype, supporting a role for *AS2* in down-regulation of *KNOX* genes.

3-81 Mutations in a MAP3K gene suppress elongation of the Arabidopsis zygote and development of its basal daughter cell into a suspensor

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Early development of Arabidopsis proceeds through a characteristic pattern of cell divisions. Upon fertilization, the zygote elongates more than 2-fold and divides transversely into a small apical and a large basal cell. The basal cell and its daughters continue to elongate and to divide transversely producing the mostly extra-embryonic suspensor. The apical cell grows in a more isodiametric way and produces the embryo proper.
We have identified mutations in a gene, designated YODA, that affect this process. Mutant zygotes elongate only about 50% before dividing. The basal cell is much smaller than in wild type and the division pattern in the basal lineage becomes irregular. As a consequence, no visible suspensor is formed, and a molecular marker for suspensor development is absent in 80% of the mutants.
Despite this early defect, YODA embryos occasionally develop into complete, viable seedlings: about 10% of the mutants germinate on soil where they grow into severely dwarfed, sterile plants.
The YODA gene encodes a MAP3K and is expressed throughout development in all tissues analyzed. All mutations we recovered are predicted to abolish or reduce kinase activity. We are in the process of analyzing the phenotypic effect of mutations in the presumptive regulatory domains of the protein. A comparison of mRNA isolated from wild type and mutant rosette leaves on microarrays showed that only relatively few genes were up- or down-regulated greater than 3-fold. We are presently searching these expression profiles for similarities to previously described signaling events.

3-82 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, the embryonic root is formed through an almost invariant sequence of cell divisions involving the uppermost suspensor cell, termed hypophysis, as well as the basal cells of the embryo proper. The hypophysis gives rise to the quiescent center and the central root cap. The adjacent cells of the embryo proper form the initials and the lateral root cap.
We have recovered mutations in a gene, designated MONOPOLE, that interfere with this process. The basal cells of mutant embryos divide aberrantly producing fewer and irregularly arranged daughters. The hypophysis cell never divides. As a consequence, no root primordium is recognizable by anatomical criteria. However, a molecular marker for the quiescent center is not absent in the mutants but rather expressed ectopically in the central cells of the embryo propper. Thus, MONOPOLE mutations do not result in a deletion but more likely in a fate-map shift.
About 20% of the mutant embryos subsequently develop into somewhat misshappen but viable seedlings. At the rosette stage, mutant plants are virtually indistinguishable from wild type.
The MONOPOLE gene encodes a protein with high similarities to transcriptional regulators of the GATA family. Consistent with this, a GUS:MONOPOLE fusion protein is found in the nucleus, and MONOPOLE fusion proteins containing a LexA DNA-binding domain can activate transcription in yeast. Preliminary results suggests that MONOPOLE transcripts are transiently expressed in the cells of the embryo proper. No expression could be detected in the suspensor and in embryos past the heart stage of development.

3-83 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 the *35S::CPC* transgenic plants generate ectopic root hairs. It indicates that CPC is a positive regulator of the hair-cells differentiation. Recent analyses showed *CPC* was preferentially transcribed in hairless cells. In addition, *CPC* was expressed in all the epidermal cells in the *cpc* mutant, while the expression level of *CPC* was decreased in the *35S::CPC* lines. Therefore, it is likely that the specific expression of *CPC* in the hairless cells is, at least partly, controlled by CPC itself. To determine the regulatory element on the *CPC* promoter, we analyzed the transgenic plants in which a series of the truncated *CPC* promoter was combined to the *GUS* gene. The GUS staining experiments revealed that the epidermis-specific transcription of *CPC* required for about 70 bp region between -267 to -336 position. This region includes two putative Myb binding sites. The epidermis-specific expression was disappeared when the mutations were introduced in these sites. We are examining interactions between the *CPC* promoter and root hair-related Myb proteins.

3-84 Functional analysis of BABY BOOM transcriptional activity

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The developmental switch from pollen to microspore-derived embryo development in *Brassica napus* was used to identify embryo-related genes that are specifically up-regulated during this fate transition. One of these genes, *BABY BOOM* (*BBM*), encodes a transcription factor belonging to the AP2/ERF family and is preferentially expressed in developing embryos and seeds. Functional analysis by ectopic expression of the *BBM* gene suggests that the protein activates signal transduction pathways leading to embryo formation from differentiated somatic cells. The nature of the pleiotropic phenotypes observed (hormone-free regeneration, altered leaf/flower morphology...) also suggests that BBM acts by stimulating cell proliferation and morphogenesis pathways.
To better understand the role of BABY BOOM during plant development, we searched for BBM-interacting proteins using the yeast two-hybrid system. BBM showed autoactivation in the GAL4-based two-hybrid screen 5' and 3' deletions of the *BBM* gene were made and tested in yeast for transcriptional activation, which allowed the identification of two strong activation domains in the BABY BOOM protein. The same constructs were analysed transiently in plant cells revealing a possible cell/tissue-specific modulation of the activation domains. To further investigate the role of each of these domains BBM deletion derivatives were placed under the control of the CaMV 35S promoter and transformed to Arabidopsis. The results of the in vitro analysis in yeast and plant cells, as well as the preliminary data on the transgenic plants over-expressing the BBM deletion derivatives will be presented.

3-85 The AtOPT3 gene, encoding an oligopeptide transporter, is essential for embryo development in Arabidopsis

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A T-DNA-tagged population of *Arabidopsis thaliana* was screened for mutations in AtOPT3, encoding a member of the oligopeptide (OPT) family of peptide transporters (Koh et al., 2002. Plant Physiol. 128: 21-29), and a recessive mutant allele, atopt3, was identified. Phenotypic analysis of atopt3 showed that most homozygous embryos were arrested at/or prior to the octant stage of embryo development and none showed the usual periclinal division leading to the formation of the protoderm. This defective phenotype could be reversed by complementation with the full-length, wild type AtOPT3 gene. A GUS fusion to DNA sequences upstream of the putative AtOPT3 ATG start codon was constructed and the expression pattern was assayed in transgenic plants. AtOPT3 was expressed in the vascular tissues of seedlings and mature plants, as well as in pollen. Consistent with the function of AtOPT3 in embryogenesis, ATOPT3::GUS expression was also detected in the developing embryos, as well as in the maternal tissues of seeds. The data argue for a critical role for peptide transport in early embryo development.

3-86 Mapping QTL for leaf morphology

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To ascertain whether intraspecific variability might be a source of information as regards the genetic controls underlying plant leaf morphogenesis, we analyzed variations in the architecture of vegetative leaves in a large sample of *Arabidopsis thaliana* natural races. A total of 188 ecotypes from the *Arabidopsis* Information Service collection were grown and qualitatively classified into 14 phenotypic classes, which were defined according to petiole length, marginal configuration and overall lamina shape. Ecotypes displaying extreme and opposite variations in the above-mentioned leaf architectural traits were crossed and their F2 progeny was found to be not classifiable into discrete phenotypic classes. Furthermore, the leaf trait based classification was not correlated with estimates on the genetic distances between the ecotypes being crossed, calculated after determining variations in repeat number at 22 microsatellite loci. Since these results suggested that intraspecific variability in *Arabidopsis thaliana* leaf morphology arises from an accumulation of mutations at quantitative trait loci (QTL), we studied a mapping population of recombinant inbred lines (RILs) derived from a Landsberg *erecta*-0 x Columbia-4 cross. 100 RILs were grown and the third and seventh leaves of 15 individuals from each RIL were collected and morphometrically analyzed. We identified a total of 16 and 13 QTL harboring naturally occurring alleles that contribute to natural variations in the architecture of juvenile and adult leaves, respectively. Our QTL mapping results confirmed the multifactorial nature of the observed natural variations in leaf architecture.

3-87 Genetic interactions and positional cloning of *HEMIVENATA*, a gene involved in venation pattern formation

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With the aim to determine the genetic basis of the sparse venation pattern found in the Eifel-5 (Ei-5) ecotype, a phenotype referred to as Hemivenata (Hve), we have studied the F_2 progeny of an Ei-5 x Ws-2 cross and found that it is inherited as a monogenic recessive trait. The reduced vascular density and number of branching points per surface unit of *hve* leaves cosegregates with an increased stem branching, an altered root waving and a low fertility, suggesting that the *HVE* gene is required for different developmental processes. Fine mapping delimited the *HVE* gene to an interval of 61 kb, located within the T8K22 BAC clone. Progress on the cloning of *HVE* and the analysis of its interactions with other genes involved in vascular development and auxin transport and/or perception will be presented.

3-88 Genetic and molecular analysis of the *ULTRACURVATA* genes

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Contrary to wild-type Arabidopsis vegetative leaves, which are flattened organs; those of *ultracurvata* (*ucu*) mutants are spirally rolled downwards and show a reduced expansion along the longitudinal axis. We have identified six *ucu* lines, whose genetic analysis indicate that they fall into two complementation groups, *UCU1*, which includes one recessive and two semidominant alleles, and *UCU2*, with three recessive alleles. Several organs in the *ucu2* mutants are helically rotated along the longitudinal axis. Double mutants involving *ucu1* and *ucu2* alleles resemble brassinosteroid response mutants, suggesting the implication of *UCU1* and *UCU2* in brassinosteroid signal transduction. Following a map-based strategy, we cloned the *UCU1* gene, which encodes a SHAGGY/GSK3-like kinase required for cell elongation. Our attempts of positional cloning of *UCU2* finally delimited a candidate region including mutations in two adjacent genes, one coding for a member of the FKBP (FK506-binding protein) family of proteins and the other for a regulatory subunit of a protein phosphatase, whose animal homologues act together with SHAGGY/GSK3 in the Wingless/Wnt signallig pathway.

3-89 Leaf morphological mutants induced by fast-neutron bombardment

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In an attempt to isolate either null or extremely hypomorphic alleles of genes required for leaf development, we performed a mutant search, screening 23,445 M_2 *Arabidopsis thaliana* individuals, the progeny of 2,931 M_1 Landsberg *erecta* (L*er*) parentals mutagenized by fast neutron bombardment, and selected plants displaying alterations in the morphology of their vegetative leaves. A total of 901 M_2 putative mutants were isolated, most of which showed unstable phenotypes that were lost after one or two generations of selfing. We subjected to genetic analysis 25 mutant lines, whose leaf phenotypes were transmitted with complete penetrance and small variations in expressivity, which fell into 9 complementation groups. Almost all of the mutations studied affect genes previously undescribed at the mutational level, their most conspicuous phenotypic traits being dentate leaf marginal configuration and reduced number of mesophyl cells (*denticulata29* and *30*), billaterally asymmetric leaves (*asymmetric leaves3*), dwarfism and circinate leaves (*ultracurvata2*), involute leaves (*incurvata8* and *15*), and small leaves (*exigua9*). Novel alleles of previously described genes, such as *CLF* and *AS1*, were also obtained.

3-90 Genetic and molecular analysis of the *ICU9* gene

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Here we describe ethyl methanesulfonate-induced alleles of the *INCURVATA9* (*ICU9*) gene, which cause several leaf abnormalities, such as organ radialization and the presence of abaxial trichomes in juvenile leaves. These morphological aberrations can be interpreted as a consequence of a partial transformation of abaxial into adaxial leaf identity, which makes the *ICU9* gene a candidate for being involved in the specification and/or maintenance of leaf dorsoventrality. In order to identify genetic operations at work during leaf morphogenesis, we crossed icu9 mutants to mutants affected in other *ICU* loci, all of which display curled, involute leaves. The synergistic phenotypes found in several of the double mutants obtained suggest that *ICU9* is functionally related to other five *ICU* genes. All these six genes seem to be essential for the leaf to properly develop. Following a map based strategy, the *ICU9* gene was found to be located within the F11A17 BAC clone. We are currently sequencing several candidate genes included in this BAC.

3-91 Positional cloning of the *ICU2* gene

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One of the largest available collections of plant morphological mutants is the Arabidopsis Information Service (AIS) Form Mutants collection. We studied 152 AIS lines already known to display abnormally shaped leaves, finding 22 that exhibited involute, upwardly curled, vegetative leaves, a phenotype that we named Incurvata (Icu). Here we present advances in a positional attempt to clone the *INCURVATA2* gene, whose recessive allele *icu2* is carried by the AIS line N329, and which was mapped near the lower telomere of chromosome 5. The *icu2* mutation causes early flowering and Apetala flowers, together with involute leaves, a phenotypic trait associated to ectopic derepression in the leaves of the *AGAMOUS* and *APETALA3* floral organ identity genes. A map-based strategy allowed us to locate the *ICU2* gene within a 60 kb interval included in the K21H1 TAC.

3-92 Genes involved in proper formation of the protoderm and epidermis

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Epidermis of higher plants plays important roles in morphogenesis, retention of water, gas exchange, and defense against pathogen. Epidermal cells are derived from a meristematic surface layer named protoderm. The protodermal cells and epidermal cells possess common characteristic features such as their anticlinal cell division and deposition of cuticle on their "outer" surfaces. We previously reported that the *abnormal leaf shape1* (*a1e1*) mutant of *Arabidopsis thaliana* is defective in surface functions of cotyledons and juvenile leaves such that the seedlings tend to dry up and cotyledons and leaves fuse to each other. The *ALE1* gene encodes a member of the subtilisin-like serine protease family and strongly expressed in endosperm cells that surrounded developing embryos (Tanaka et al., 2001), suggesting that a protein processing.

3-93 Tissue-specific ectopic expressions of the “moving” putative transcription factor SHORT-ROOT: Studying the positional information in the root radial pattern?

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During post-embryonic development the root radial pattern is formed by asymmetric divisions of a set of stem cells (initials) located in the apical meristem. Such "asymmetries" are primarily dependent on positional information, but little is known about the actual signaling mechanism responsible for it.
The *short-root* (*shr*) mutant has only one ground tissue layer (cortex) in its root, instead of the two found in wild-type (cortex and endodermis). The SHR gene belongs to a family of plant-specific putative transcription factors (GRAS) and in the root it is expressed in the stele, but not in the endodermis nor in the endodermis/cortex initials. Surprisingly, it has been shown (1) that the SHR protein moves from the stele into all the "first-neighbor" cells, *i.e*. the endodermis tissue layer, the endodermis/cortex initials and the quiescent center. No SHR protein is detectable in any other tissue more distant from the stele. Nothing is known about the mechanism responsible for such "first-neighbor" movement. Moreover, ectopic expression of *SHR* can result in the alteration of both cell fates and cell division pattern in the root. Both tissue-specific regulation of SHR movement and competence to respond to it with cell divisions or endodermis fate acquisition seem to be part of the mechanism regulating the root radial patterning.
Here we present preliminary data about tissue-specific ectopic expressions of the protein fusion SHR-GFP. Aspects of radial pattern modification, cell fate acquisition and SHR-GFP protein movement will be discussed.

3-94 Genetic analysis reveals the relationship between sterol biosynthesis enzymes in embryonic development of Arabidopsis

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Plant cells contain a complex mixture of phytosterols, the most abundant species being sitosterol and campesterol. Whereas campesterol is the precursor to the brassinosteroids (BRs), which are established as phytohormones in post-embryonic growth, so far no plant steroid products are specifically implicated in embryogenesis. The identification of the Arabidopsis *fackel* (*fk*) mutants provided the first genetic evidence that steroids in addition to BRs are required for embryonic growth and patterning. *FK* encodes a sterol C-14 reductase that acts upstream of all known enzymes corresponding to BR biosynthesis mutants. In genetic screens for *fk*-like mutants we identified two additional genes encoding sterol biosynthesis enzymes in the same upstream branch: *CEPHALOPOD* (*CPH*), a C-24 methyl transferase and *HYDRA1* (*HYD1*), a C-8,7 isomerase. Similar to *fk* mutants, *cph* and *hyd1* mutants show embryonic cell proliferation and cell expansion defects, as well as patterning defects such as multiple shoot meristems. Double mutant analysis revealed that *FK* and *HYD1* act together whereas *CPH* acts independently of *FK* and *HYD1* to produce essential sterols. We also examined the relationship to the BR biosynthesis pathway, and found that the BR biosynthesis gene *DWF1* acts independently of *FK* while the BR receptor gene *BRI1* acts downstream of *FK* to promote post-embryonic growth. Experiments using the azasterol inhibitor of sterol C-14 reductase corroborate these findings. We postulate that the sterol biosynthesis pathway represents a complex interplay of enzymatic functions leading to the production of phytosterols that play as yet unidentified roles in embryonic development.

3-95 Characterization of *tonsoku* mutant with abnormal shoot apical meristem and root apical meristem

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The recessive *tonsoku (tsk),* mutant shows reduced growth in root and fasciation in aeral part of the plant. Microscopic analyses of the *tsk* mutant showed disorganized structure of the root tip and flat and large shoot apical meristem (SAM), suggesting that *tsk* affects structures of both RAM and SAM. Expression of *WUS* in SAM of the *tsk* mutant was altered compared to those in SAM of other fasciation mutants. SAM of the *tsk* mutant contained few separated areas of *WUS* expression.
Abnormal cell division planes were occasionally detected in the embryo and root of the *tsk* mutant, suggesting that the function of TSK is important in the determination of the direction of cell division. The *tsk* mutant was tagged with T-DNA, and the *TSK* gene was found to encode a large protein with 1,311 amino acid residues. The N-terminal part of TSK contains sequences similar to LGN repeats in PINS of *Drosophila* which is involved in asymmetric cell division. The TSK also contains leucine rich repeats (LRRs) at the C-terminas. These sequences are probably involved in the interaction with other proteins.

3-96 Genetic studies of the early first steps of *Arabidopsis thaliana* embryogenesis

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The double fertilization of flowering plants has been described more than one century ago, but the development initiation mechanisms of the zygotic embryo and endosperm by fertilization are not yet resolved. In some species, *in vitro* fertilization and somatic embryogenesis implicated external signals exerced on the embryo. The aim is to know wether these type of signals are active inphysiological condition during Arabidopsis zygotic embryogenesis. Genetic approach for the analysis of the Arabidopsis development, based on gametophytic mutations screen, have shown that transcription factors of the *Polycombs* group are involved in the repression of the central cell development before fertilization. In order to characterize essential genes activating the seed development, a forward genetic approach is used. Several *embryo-defective* (*emb*) mutants of the INRA of Versailles Arabidopsis T-DNA insertion collection have been isolated. These mutations are sporophytic and recessive and are conserved by the heterozygous plants. Among *emb* mutants, those arrested at the zygote elongation stage are called *zygote* (zyg), and those arrested at the first assymetric division of the zygote are called *cyclops* (*cyl*). Each mutant line is phenotypically described for the development of the endosperm, embryo and integuments. The fine phenotypic description of *zyg* and *cyl* mutants can reveal what interaction exists between the seed compartments. The mutant phenotype is observed in very young silique in order to determine if the paternal allele of the gene is silenced or not during the first steps of embryogenesis. Molecular characterization of the tagged mutants, allelic test and screen are initiated.

3-97 EMB506: A plastidic protein involved in embryogenesis and plastid maintenance

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The development of the young Arabidopsis embryo can be genetically investigated by screening mutant collections for *emb* (embryo-defective) mutations. Numerous mutants have been isolated, allowing an estimatation of about 500 to 1000 essential and non-functionnally redundant genes in Arabidopsis. Among them, the *EMB506* gene has been shown to encode a protein containing 5 ankyrin repeats. The globular mutant phenotype does not suggest any function for the EMB506 protein. GFP protein fusion experiments have demonstrated that EMB506 was targeted to plastids, like about 25% of the ORF disrupted in *emb* mutants. Partial complementation of homozygous *emb506* mutants using a seed-specific promoter showed that EMB506 was also necessary during adult phases of the plant, since the lack of the protein induces a chlorotic phenotype of stems and inflorescence.
The ankyrin repeats domain suggested that a partner could interact with EMB506. One candidate was isolated using the two-hybrid system. The corresponding full-length cDNA is predicted to code for a protein containing an ankyrin repeats domain very similar to EMB506, and a plastid-targeting signal. No similarity to any known protein could be detected on the N-terminal part of the predicted protein. Transgenic plants containing antisens construct to this cDNA presented a chlorotic phenotype of rosette leaves and inflorescence. Together, these results suggest that EMB506 is essential not only during embryogenesis but also after germination, and acts in the plastid through an interaction with another ankyrin repeat containing protein.

3-98 Purification of KNOLLE-containing protein complexes

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The cytokinesis-specific syntaxin KNOLLE plays an essential role in the fusion of membrane vesicles in the plane of cell division to build the new membrane compartment called the cell plate. In homozygous mutant embryos, KNOLLE-positive vesicles still reach their destination but fusion is impaired. A few proteins have been identified as putative KNOLLE interactors, using *in-vitro* binding or yeast two-hybrid assays and co-localisation: KEULE, a member of the Sec1 family (Assaad et al. 2001), the t-SNARE AtSNP33 (Heese et al. 2001), and the SNARE NPSN11 (Zheng et al. 2002). Considering the complex machinery of membrane fusion in other well-studied systems, many more proteins can be expected to act together with KNOLLE in the process of vesicle fusion during cytokinesis. To identify additional KNOLLE-interacting proteins we have taken a biochemical approach of purifying protein complexes from Arabidopsis callus culture.

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3-99 Synergistic activation of seed-storage protein gene expression in Arabidopsis by ABI3 and two bZIPs related to OPAQUE2

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In Arabidopsis, major seed storage protein genes are expressed at early and mid stages of maturation under a tight temporal and tissue-specific regulation. Here, we present several lines of evidence on support that the two transcription factors characterised in this study participate in this regulation. First, they are structurally very closely related to Opaque2-like factors involved in the regulation of seed storage protein genes in cereal species. Moreover, they are transcription activators, expressed in the seed at the maturation phase, and bind to regulatory sequences in SSP gene promoters. Finally, over-expression of these factors, in conjuction with ABI3, induces the ectopic expression of SSP genes.

3-100 The MADS box gene *AGL42* is expressed in the quiescent center and maintains root meristem organization

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The quiescent center (QC) is a population of slowly cycling stem cells at the heart of the root meristem with special properties, including pluripotency and the ability to generate intact roots upon explantation. We are taking advantage of an enhancer trap expressed exclusively in these cells, in an effort to understand QC function at the molecular level. Cloning of the locus has led to the isolation of *AGL42*, a novel member of the MADS box transcription factor family. RTPCR and transgenic plants bearing a promoter-GFP fusion have confirmed the highly specific expression of *AGL42* in the QC of primary and lateral roots. Two independent hypomorphic alleles and plants expressing AGL42(RNAi) reveal a defect in root meristem organization. Preliminary results assign a role for *AGL42* in maintaining the structure and developmental function of the root meristem.

3-101 Biogenesis of thylakoid membranes in Arabidopsis

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Thylakoid membranes of higher plants are complex structures. The majority of their constituent polypeptides is organized into four major protein complexes, the photosystems I and II, the cytochrome b/f complex and the ATP synthase. Each of these complexes may in addition contain non-proteinaceous cofactors like pigments or metal ions. The genetic and structural complexity of these protein complexes suggests that their biogenesis should require various regulatory and auxiliary factors which act at different levels of the gene expression pathways and which are involved in assembly processes. To identify those factors a forward genetic approach was pursued with *Arabidopsis thaliana* as the experimental model organism. Photosynthesis-deficient mutants were isolated using the high chlorophyll fluorescence (hcf) phenotype as a criterium of selection. These hcf mutants were found to be specifically deficient in individual protein complexes or showed pleiotropic defects. The affected genes were isolated by the aid of T DNA insertions or by positional cloning. In the presentation, photosystem II will be used as an example to illustrate which types of genes have been isolated by this approach and how the mechanisms of fucntion of the isolated genes are being elucidated.
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3-102 KNAT1 overexpression interferes with primary root meristem development in *Arabidopsis thaliana*

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Homeodomain proteins are transcription factors that trigger the expression of specific target genes and control important differentiation processes in a variety of organisms.
In plants, the KNAT-genes, belonging to the Knotted1 homeodomain gene family, have been isolated in *Arabidopsis*. KNAT1, the best characterized member of this gene family, has been shown to play an important role in shoot meristem organisation (Lincoln, 1994). We are investigating the role of the members of the KNAT gene family in establishing and maintaining cell identity in the *Arabidopsis* root and have shown that misexpression of these proteins has the potential to perturb cellular interactions and modify plant development.
We have generated plants expressing KNAT1 fused to the yellow fluorescent protein (YFP) under the control of a constitutive plant promoter.
Misexpression of the KNAT1 homeodomain protein was shown to interfere with normal root development after germination. KNAT1 misexpression perturbed the balance of cell division and elongation that normally maintains the highly organized and dynamic root architecture. We have found strong evidence that overexpression of the homeodomain protein interferes with plant hormone mediated organisation and maintenance of the root meristem. This study outlines the first direct evidence for the role of the KNAT homeodomain genes in *Arabidopsis* root development.

4-01 SCF-mediated Aux/IAA stability and the auxin response

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Our understanding of auxin signalling and auxin-induced gene expression is based largely on the mutational and molecular characterisation of three groups of proteins; the Auxin Response Factors (ARFs), the Aux/IAAs, and components of the ubiquitin-mediated proteolytic pathway. ARFs and Aux/IAAs are both large families of transcription factors that interact to bring about the expression or repression of auxin-inducible genes. It seems that regulated degradation of the remarkably short-lived Aux/IAA proteins is essential to the regulation of these activities. Dominant, gain-of-function mutations affecting the destabilisation sites of several Aux/IAAs result in severe and pleiotropic auxin-related phenotypes, which are a consequence of the increased stability of those Aux/IAA proteins. A similar range of phenotypes is observed in the *axr1* and *tir1* loss-of-function mutants. TIR1 forms part of the ubiquitin-ligase complex called SCF^{TIR1} responsible for recruiting and ubiquitinating target proteins, while AXR1 is part of an enzyme cascade that affects the activity of SCF^{TIR1}. Recently, we, with our collaborators, have shown that SCF^{TIR1} interacts with the Aux/IAA proteins AXR2 and AXR3, and that the AXR1/ SCF^{TIR1} pathway is responsible for the turnover of Aux/IAA proteins. Importantly, auxin accelerates the degradation of Aux/IAAs by promoting their interaction with SCF^{TIR1}. This last finding suggests a model in which auxin-induced changes in the abundance of Aux/IAA proteins are responsible for the induction of genes in response to auxin. Using both genetic and biochemical approaches we are now trying to understand exactly how auxin stimulates the Aux/IAA-SCF interaction and how the resulting changes in Aux/IAA levels affect the the transcription of auxin-regulated genes.

4-02 BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation in Arabidopsis

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Plant steroid hormones brassinosteroids (BRs) play important roles throughout plant growth and development. Plants defective in BR biosynthesis or perception display pleiotropic dwarf phenotypes, including short hypocotyls and leaf petioles, epinastic and dark-green leaves. Unlike the classical model of animal steroid action in which steroids directly bind nuclear receptors to activate gene expression, BRs signal through a plasma membrane localized, leucine-rich repeat (LRR) receptor kinase BRI1. BIN2, a glycogen synthase kinase-3 (GSK-3), is implicated in BR signaling as a negative regulator, since gain-of-function *bin2-D* mutants display BR-insensitive dwarf phenotypes. To identify positive signaling components downstream of BRI1, we performed a *bri1* suppressor screen and identified a semidominant mutant *bes1-D*. *bes1-D* not only completely suppresses *bri1* dwarf phenotypes, but also exhibits constitutive BR response phenotypes including long and bending petioles, curly leaves, and constitutive expression of BR-response genes. BES1 protein accumulates in the nucleus in response to BRs in a BRI1-dependant manner, while the mutant bes1 protein accumulates in the nucleus at high levels even without the hormone treatment. We found that *bes1-D* also completely suppressed *bin2-D* dwarf phenotypes, indicating that BES1 acts downstream of BIN2. BES1 interacts with and is phosphorylated by BIN2. In addition, BR-induced BES1 accumulation is impaired in gain-of-function *bin2-D* mutant, demonstrating that BIN2 negatively regulate BES1 protein level. These results establish a signaling cascade for BRs with similarities to the Wnt pathway, in which signaling through cell surface receptors leads to inactivation of a GSK-3 allowing accumulation of a nuclear protein that regulates target gene expression.

4-03 Dissection of guard cell ABA signal transduction mechanisms

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Guard cells have become a well-suited model system for dissecting early signal transduction mechanisms. Previous pharmacological research suggested that type 2A protein phosphatases (PP2As) act as both negative and positive regulators of ABA signaling. To dissect the molecular mechanisms responsible for these proposed counteracting PP2As, gene chip experiments were performed with Arabidopsis guard cell RNA together with degenerate oligo-based PCR screening of Arabidopsis guard cell cDNA libraries. A T-DNA insertion allele in one of guard cell-expressed PP2A genes, *rcn1*, was obtained and showed ABA insensitivity in stomatal movements and anion channel activation. Calcium imaging analyses show a reduced sensitivity of ABA-induced cytosolic Ca2+ ([Ca2+]cyt) elevations in *rcn1*, whereas mechanisms downstream of [Ca2+]cyt increases show wild-type responses, suggesting that RCN1 functions upstream of [Ca2+]cyt increases. *rcn1* shows ABA insensitivity in ABA inhibition of seed germination and ABA-induced gene expression. The PP1/2A inhibitor, okadaic acid, phenocopies the *rcn1* phenotype in wild-type plants. These data show that RCN1 is a positive transducer of early ABA signaling. Furthermore, detailed analyses of gene chip experiments with Arabidopsis mesophyll and guard cell RNA show that there are about 300 guard cell-preferential genes regulated by ABA, which will lead to identification of molecular players of ABA signaling. Hyperpolarization-activated Ca2+-permeable channels (ICa) were identified as a component of ABA signaling. ROS were shown to activate ICa channels in Arabidopsis guard cells. Data will be presented analyzing NADPH oxidase disruption mutations that suggest a central role for these genes in ABA activation of ICa channels and stomatal closure.

4-04 Involvement of the Arabidopsis Response Regulator ARR2 in stress and hormone signaling

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Within the last years it became evident that elements of His-to-Asp two-component signaling pathways are conserved in higher plants, yet only limited information is available about their specific functions and cross-talk properties. During our studies we characterized the B-type response regulator 2 (ARR2) as a transcription factor, which binds to regulatory sequences within the promoters of nCI genes and genes regulated by ethylene. Characterization of an ARR2-knockout line revealed an altered phenotype in respect to vegetative growth and stress resistance. Moreover, ARR2-knockout seedlings exhibit reduced sensitivity to cytokinin and ethylene but not to any other tested hormone. Transgenic plants overexpressing a mutant form of ARR2, in which the phosphorylation of the conserved Asp in the receiver is omitted, showed an aberrant phenotype in vegetative growth as well. Phosphotransfer assays in plant and yeast cell-free systems, yeast two- and three-hybrid interaction analyses, cell biological approaches and biochemical studies suggest that the membrane-associated cytokinin and ethylene receptors (CRE1, ETR1) act on the transactivation capacity of nuclear ARR2 very likely via a shuttling phosphotransfer protein (AHP, probably AHP2). Taken together, our data suggest that, in addition to the "classical" CTR1-dependent pathway, there exists a second ethylene signaling mechanism in higher plants, which consists of an ETR1/AHP/ARR2 phosphorelay. Furthermore, the observation that, for instance, AHP2 interacts with several histidine kinases (e.g. ETR1, CRE1, CKI1) and response regulators (e.g. ARR2, ARR9) suggests that plant two-component systems form a complex signaling network, which integrates different endogenous and exogenous signals to control plant growth and development.

4-05 Two-component circuitry in Arabidopsis cytokinin signal transduction

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Cytokinins are essential plant hormones that control cell division, shoot meristem initiation, leaf and root differentiation, chloroplast biogenesis, stress tolerance, and senescence. Together with another plant hormone auxin, cytokinins can reprogram terminally differentiated leaf cells to stem cells and support shoot regeneration indefinitely in plant tissue culture. Thus, cytokinins are master regulators of plant growth and development that is highly plastic and adaptive as well as remarkably resilient and perpetual. Recent rapid advances have discovered hybrid histidine protein kinases (AHKs) as cytokinin receptors, histidine phosphotransfer proteins (AHPs), and nuclear response regulators (ARRs) as transcription activators and repressors in the Arabidopsis cytokinin signal transduction pathway. Similar components are also found in maize, suggesting a conservation of the cytokinin signaling mechanism in plants. There are four major steps in the cytokinin phosphorelay: AHK sensing and signaling, AHP nuclear translocation, ARR transcription activation, and a negative feedback loop through cytokinin-inducible ARR gene products. Analyses of mutants and transgenic tissues and plants support the importance of this central signaling pathway in diverse cytokinin responses, including promoting shoot meristem proliferation and leaf differentiation, as well as delaying leaf senescence.

4-06 The RKS family of transmembrane receptor kinases

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The Receptor Kinases like SERK (RKS) family of transmembrane receptor kinases is represented by 15 different members in *Arabidopsis thaliana*. Together with a small related group of 3 Extracellular Like SERK (ELS) proteins they are involved in transmitting extracellular signals towards intracellular compartments. Specific features of the predicted RKS proteins consist of a signal sequence, an extracellular leucine zipper domain, a putative ligand binding domain containing only 5 leucine rich repeats (LRRs) surrounded by conserved cysteine residues and a single LRR motif at the intracellular C-terminus. This last domain is unique for the RKS family and is absent in all other classes of plant transmembrane receptor kinases. ELS proteins share all the conserved extracellular domains of RKS, but lack transmembrane and intracellular domains.
The developmental function of all members of these receptors in *Arabidopsis thaliana* was characterized using transgenic plants overexpressing or co-suppression RKS and ELS genes.

4-07 Molecular and physiological analysis of a novel hormone response mutant

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Many of the components of the Abscisic acid (ABA) signal transduction pathway, including the ABA receptor, have still to be identified. The identification of mutants with altered responses to ABA, however, has led to substantial progress in recent years. Genetic screens based on the inhibition of seed germination by exogenous ABA have led to the isolation of several Arabidopsis mutants with altered ABA responsiveness. The *ABA insensitive (abi)* mutations *abi1* to *abi5* result in reduced sensitivity of seed germination to exogenous ABA, whilst the *enhanced response to ABA (era)* mutants have increased sensitivity to ABA and germinate much later than WT. In addition to ABA, brassinosteroids have also been shown to play a role in germination, brassinosteroid mutants are more sensitive to ABA with respect to inhibition of germination. There is evidence that brassinosteroids act in parallel, or downstream of GA, to counter the effect of ABA and stimulate seed germination.
Here we describe the isolation and characterisation of a novel Arabidopsis recessive dwarf mutant that has altered responses to both ABA and epi-brassinosteroid, conferring increased sensitivity to both phytohormones in germination and root elongation assays. The T-DNA has inserted in a novel plant gene which in database searches shows highest homology to mammalian and fruit-fly heparin sulphate polymerases involved in the production of heparin sulphate proteoglygans (HSPGs). Membrane bound HSPGs are proposed to act as co-receptors and/or internalisation receptors, for growth factors and have not been described in plants before.

4-08 Signaling in the Arabidopsis meristem: BAM, BAM, and a Club

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All aboveground organs in plants are initiated at shoot and floral meristems. For meristems to continually initiate new organs, they must maintain a population of undifferentiated stem cells and direct appropriately positioned progeny cells toward differentiation and organogenesis. The WUSCHEL (WUS) transcription factor is key in specifying stem cell fate and its expression domain determines the number of stem cells present at the meristem. The expression pattern of *WUS* is negatively regulated by three genes, *CLAVATA1* (*CLV1*), which encodes a receptor serine kinase; *CLV2*, which encodes a receptor-like protein; and *CLV3*, which encodes a small, proteinacious ligand. Plants mutated at a *CLV* locus lose *WUS* repression and accumulate extra stem cells. Because most *clv1* mutant alleles are dominant negative and are more severe in phenotype than the *clv1* null allele, we hypothesized that the dominant negative *clv1* alleles were interfering with additional receptor kinases. To test this hypothesis, we isolated loss of function alleles for two Arabidopsis genes (*BIG APICAL MERISTEM 1* [*BAM1*] and *BAM2*) related to *CLV1*. Plants homozygous for the loss of function alleles of *BAM1* or *BAM2* have no obvious phenotypic defects, however mutations at the *BAM* loci enhance the phenotypic defects of *clv1* null alleles. This result supports the hypothesis that BAM1 and BAM2 have functional overlap with CLV1. In addition, *bam1 bam2* double mutant plants have a number of non-meristem phenotypes suggesting that these proteins may be acting redundantly and are likely involved in a number of other signal transduction pathways.

4-09 Genetically engineered Arabidopsis plants with a reduced cytokinin content reveal regulatory functions of cytokinins in development

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In order to obtain information about processes for which cytokinins are the rate-limiting and possibly regulatory molecules we have engineered transgenic plants with a reduced cytokinin content. Arabidopsis plants expressing any one of six cytokinin oxidase (*AtCKX*) gene family members showed distinct developmental alterations of the shoot and root. Shoots of *AtCKX* transgenics plants had a smaller apical meristem, a slowed formation of leaf primordia, a reduced number of leaf cells, a reduced vasculature and flowered later. Visual leaf senescence did not occur earlier in *AtCKX* overexpressers which suggests that lack of cytokinins is not a physiological signal that triggers the onset of senescence. Root growth was enhanced in the *AtCKX* expressers, owing to a more rapid elongation of the primary root and lateral roots, an increased formation of lateral roots and an increased number of adventitious roots. The root meristems were larger than in wild type plants, and the number of columnella tiers and of cells in individual cell files of the cell division zone was higher. This indicated that cytokinins regulate the numbers of divisions of cells before they leave the meristem. In summary, the results proved that cytokinins are a limiting factor for cell division activity in the shoot meristem, are required for leaf cell formation and have a negative regulatory function in the root. *Promoter::GUS* fusion genes indicated differential regulation of gene family members. Regulation of the local endogenous cytokinin content is a promising tool to regulate plant organ growth and alter biomass distribution.

4-10 Auxin has dramatic and dynamic effects on gene expression in Arabidopsis

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The plant hormone auxin has been implicated in diverse aspects of plant growth and development. Rapid and specific changes in gene expression are associated with auxin treatment and several auxin induced gene families have been described. In this report we present an analysis of auxin-dependent gene expression using Arabidopsis Affimetrix Gene Chip technology. Columbia seedlings were treated with auxin during 0, 30 min, 1, 4, 12 and 24 hours. cRNA probes were generated and hybridizations to the Affimetrix Gene Chips were performed. We also examined the gene expression profile of the axr1-12 mutant. The AXR1 protein is a component of the ubiquitin-mediated proteolytic pathway which is involved in the degradation of repressors of auxin response. According to our results, auxin has dramatic and dynamic effects on gene expression. In wild type plants an important number of genes are induced or repressed 2 fold or more at some time point after auxin treatment. Surprisingly, in the mutant a larger number of genes are induced. The induced genes for wild type and mutant also showed differences in the kinetics of the response as well as the identity of the genes involved, supporting the idea that the ubiquitin-mediated proteolytic pathway is essential to trigger and regulate a normal gene induction in response to auxin. The comparison of the basal gene expression profiles (seedlings not treated with auxin) showed an extensive level of gene repression in axr1-12. We also identified genes that might be under the regulation of the ubiquitin-mediated proteolytic pathway.

4-11 Signals Involved in *Arabidopsis thaliana* resistance to cabbage looper caterpillars, *Trichoplusia ni*, induced by virulent and avirulent strains of the phytopathogen *Pseudomonas syringae*

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Plants have evolved different but interacting strategies to defend themselves against herbivorous insects and microbial phytopathogens. We used an Arabidopsis/*Pseudomonas syringae* pathosystem to investigate the impact of pathogen-induced defense responses on cabbage looper (*Tricoplusia ni*) larval feeding. Arabidopsis mutants (*npr1, pad4, eds5, eds16*) or transgenic plants (*nahG*) that are more susceptible to microbial pathogens and which have reduced levels of salicylic acid, exhibited reduced levels of feeding by cabbage loopers. Conversely, Arabidopsis mutants that are more resistant to microbial pathogens and which have elevated levels of SA (*cpr1* and *cpr6*) exhibited enhanced levels of cabbage looper feeding. These experiments suggested an inverse relationship between SA levels and insect feeding. However, In contradiction to these results, cabbage looper larvae had less weight gain on wild-type Arabidopsis plants that were infected with *P. syringae* strains carrying the avirulence genes avrRpt2 or avrB , conditions that elicit a hypersensitive response (HR) and high levels of SA accumulation. When challenged by the avirulent *P. syringae* strains, *nahG* and *npr1* (which fail to develop an HR), but not *pad4* plants (which does develop an HR), fail to show the increased resistance to insect feeding exhibited by wild-type plants. Surprisingly, wild-type plants as well as *nahG* and *npr1* plants treated with a virulent *P. syringae* strain become more susceptible to cabbage loopers. This strongly indicates a different systemic response induced by virulent pathogens that is SA indepedent.

4-12 *GCR1*, the gene encoding the heterotrimeric G-protein coupled receptor of Arabidopsis is cell cycle regulated and its overexpression increases cell division, abolishes seed dormancy and shortens time to flowering

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Signaling through heterotrimeric G-proteins is a highly conserved mechanism responsible for transducing extracellular signals in diverse eukaryotes. G-protein-coupled receptors (GPCRs) are the initial components of this pathway. Activation of GPCRs results from ligand binding and triggers the initiation of a cascade of events within the cell, resulting in changes in cellular functions including the activation of many genes. The signal transduction is mediated by the a-subunit or the bg complex of the G-protein. In plants, several studies have implicated Ga subunits in responses to hormones, light and pathogen resistance. No function has as yet been assigned to the only identified Arabidopsis receptor homologue (GCR1). We observed that expression of GCR1, is modulated during the cell cycle and during plant development. Overexpression of GCR1 in tobacco (Nicotiana tabacum) BY-2 cells caused an increase in thymidine incorporation and in the mitotic index of aphidicolin synchronized cells. Overexpression of GCR1 in Arabidopsis caused two remarkable phenotypes: seed dormancy was abolished and time to flowering was reduced. Molecular markers of these two developmental processes (phosphatase PP2A and MYB65 in germination, LFY during flowering) were upregulated in GCR1 overexpressors.

4-13 Patatin like phospholipase A genes from Arabidopsis are involved in auxin signal transduction and their transcription responds to biotic and abiotic signals

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The gene family of patatin related phospholipase A (AtPLAs) in Arabidopsis comprises 10 genes of which 9 have been isolated as cDNA´s. One of the expressed proteins was characterized and has PLA1/PLA2 activity which is inhibited by a number of PLA2 inhibitors (PACOCF3, AACOCF3, NDGA, ETYA, HELSS). HELSS and ETYA also inhibited effectively in vivo the hypocotyl elongation of etiolated Arabidopsis seedlings. Accumulation of GUS by the auxin-inducible promoters DR5 was also inhibited by these inhibitors. The subcellular localization of four hybrid GFP-PLA proteins was cytosolic when transiently expressed in tobacco leaf cells. Taken together, this suggests an involvement of PLA enzymes in auxin signal transduction, as PLA activity becomes rapidly activated by auxin and PLA inhibitors act on PLA activation (Plant J. 16:601, 1998) and on downstream processes such as elongation and gene activation. When the promoter activities of several PLA genes were investigated as GUS constructs in transgenic Arabidopsis, evidence for crosstalk with other signalling pathways was obtained. The AtPLAIIA promoter responded to SA, bion, ACC, and JA by up-regulation of the GUS gene linked to it. This points to an involvement in systemic acquired resistence and wounding. With the same approach abscisic acid and drought were shown to increase the promoter activity of the AtPLAIVC gene.

4-14 Cloning of a putative ABA-8´ hxydroxylase

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Abscisic acid (ABA) is one of the five classical phytohormones and participates in seed development, dormancy and the plant reaction towards water stress. Whereas the biosynthesis of ABA has been well characterised in the last years, little is known about its catabolism, which is thought to be involved in the regulation of ABA content.
We have cloned a cytochrome P450 monooxygenase. Expression studies revealed an up-regulation by exogenous ABA. This suggests that the cloned monooxygenase might be the ABA-8´ hydroxylase catalysing the conversion of ABA to phaseic acid, the first and commited step of ABA catabolism. The functional expression of the putative ABA-8´ hydroxylase in yeast and *Arabidopsis thaliana* L. is in progress.

4-15 Expression of *Pti4* gene confers constitutive ethylene response in Arabidopsis

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Pti4 is a tomato (*Lycopersicon esculentum*) transcription factor that belongs to the ERF (ethylene-responsive element binding factor) family of proteins. It interacts with the Pto kinase in tomato, which confers resistance to the Pseudomonas syringae pv tomato pathogen that causes bacterial speck disease. To study the function of Pti4, transgenic Arabidopsis plants were generated that expressed tomato Pti4 driven by the strong constitutive promoters, cauliflower mosaic virus 35S and tCUP. Global gene expression analysis by Affymetrix GeneChip indicated that expression of Pti4 in transgenic Arabidopsis plants induced the expression of GCC box-containing PR genes. We also demonstrated that Pti4 enhanced GCC box-mediated transcription of a reporter gene. The data suggests that tomato Pti4 could act as a transcriptional activator to regulate expression of GCC box-containing genes. Furthermore, we show that the expression of tomato Pti4 in transgenic Arabidopsis plants produced a phenotype similar to that seen in plants treated with ethylene, thus providing evidence that the Pti4 gene is involved in the regulation of a subset of ethylene-responsive genes containing the GCC box.

4-16 Overexpression of the PERK1 receptor kinase in *Arabidopsis thaliana* L. leads to an increased growth phenotype

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Receptor-like protein kinases (RLKs) play a fundamental role in the perception of external stimuli and the transmission of the stimulus through a signalling cascade to elicit appropriate cellular responses. PERK1 (Proline Extensin-like Receptor Kinase 1) was originally isolated from *Brassica napus* and constitutes a novel class of plant receptor-like protein kinases with 14 related members identified in the Arabidopsis genome. The PERK1 protein consists of a proline-rich extracellular domain showing sequence similarity to extensins, a unique transmembrane domain and a catalytic domain with serine/threonine kinase activity. Using biolistic bombardment, a PERK1-GFP fusion was found to be strictly localized to the plasma membrane of onion epidermal cells as predicted for an RLK. The role of PERK1 in mediating plant defense responses was investigated through expression studies following various treatments. PERK1 mRNA levels were found to rapidly accumulate after various wounding stimuli and increase moderately in response to fungal pathogen treatment. More recently, transgenic studies in *Arabidopsis thaliana* (Col-0) suggest that PERK1 may also have a role in plant development. The overexpression of the PERK1 cDNA under the control of the strong 35S cauliflower mosaic virus (CaMV) promoter resulted in several heritable changes in growth such as increased height, secondary branching and seed production compared to the wild-type Arabidopsis plants.

4-17 Investigating the role of cytosolic free calcium in Salicylic Acid mediated disease resistance in Arabidopsis

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The endogenous accumulation of salicylic acid (SA) is a fundamental requirement for the establishment of effective plant defence mechanisms when challenged with pathogens. Using transgenic *Arabidopsis thaliana* seedlings expressing the Ca^{2+} -sensitive photoprotein aequorin, we previously reported a rapid and transient increase in cytosolic free Ca^{2+} ($[Ca^{2+}]_c$) upon application of exogenous SA.
Biochemical characterisation of the SA-induced $[Ca^{2+}]_c$ increase suggests that the majority of the response is derived from intracellular Ca^{2+} stores, with the likelihood that SA triggers Ca^{2+} induced Ca^{2+} -release. We now investigated the role of the SA-induced $[Ca^{2+}]_c$ increase in SA mediated signal transduction. We have isolated two mutants which lack the wild type SA-induced $[Ca^{2+}]_c$ increase and report that both mutations are not due to a general deficiency in Ca^{2+} based signalling. Preliminary evidence suggests a role for the SA-induced $[Ca^{2+}]_c$ increase in the regulation of *NPR1* expression since a reduction in *NPR1* mRNA levels was observed in the 7-102 mutant line following treatment with SA. Currently we are investigating the role of the SA-induced $[Ca^{2+}]_c$ increase on other early SA-inducible genes in both wild type and mutants. Further characterisation of the mutants is underway and will prove invaluable in identifying the components or events that cause the SA-induced $[Ca^{2+}]_c$ transient, thereby aiding in the understanding of the role of $[Ca^{2+}]_c$ in SA-mediated signal transduction.

4-18 *IGL* gene promoter analysis in transgenic *Arabidopsis thaliana*

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The maize indole-glycerolphosphate lyase (*IGL*) gene belongs to the secondary metabolic pathway and is inducible by an elicitor. Igl expression is part of the so-called tritrophic interaction between plant, herbivore and parasite of the herbivore in maize. We analysed the *IGL* sequence upstream of the coding region by means of GUS-reporter constructs in transgenic *Arabidopsis thaliana*. Distinct expression patterns are observed for the different constructs (0,36 kb to 5,3 kb of upstream sequence). A specific marking of distal leaf-tip cells was displayed by several constructs. The influence of a 26 bp motif which is shared by both, *IGL* from maize and ACC-synthase of Arabidopsis has been investigated.

4-19 Involvement of mitogen-activated protein kinase (MAPK) activity in the jasmonate-signalling pathway

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Jasmonates (JAs) including jasmonic acid (JA) and its methyl-ester methyl jasmonate (MeJA) are fatty acid derivatives that act as plant hormones. They are implicated in plant resistance to insects and fungal pathogens. The signal transduction cascade, from detection of JAs by the plant to gene expression and production of defensive compounds, is largely unknown. Mitogen-activated protein kinases (MAPKs) are well established as signalling intermediates in mammalian and yeast signal transduction cascades and have been implicated in plant signalling pathways to a diverse range of biotic and abiotic stimuli. Here we report the activation of a 45-kD protein able to phosphorylate myelin basic protein (MBP), which is characteristic of MAPKs, in response to MeJA treatment. This data points towards a positive role for kinase activity in this signalling pathway.
Four MeJA signalling mutants have been published which are deficient in MeJA induced responses. *jin1*, *jin4* and *jar1* show reduced MeJA inhibition of root growth and altered expression of MeJA induced genes, while *coi1* is severely affected in all known MeJA responses. Different activation patterns of the putative MAPK were seen in the four MeJA signalling mutants of Arabidopsis. From this data we propose a model for MeJA signalling in relation to *JIN1*, *JAR1*, *JIN4* and *COI1* involving a MAPK cascade.

4-20 A novel family of calmodulin-binding transcription activators in multicellular organisms

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Screening of cDNA expression libraries with labelled calmodulin as a probe resulted in the isolation of a cDNA encoding a protein that we designated CAMTA (for calmodulin-binding transcription activator). The Arabidopsis genome contains 6 members of this gene family (AtCAMTA1-6), all containing a distinct calmodulin-binding domain, a DNA-binding domain and ankyrin-repeat motifs. Based on domain organization and sequence homologies, we identified members of this protein family also in the genomes of human, fly, and nematode but not in yeast or prokaryotes. The calmodulin-binding domain was mapped, and a synthetic peptide of this region was shown to form a complex with calmodulin in the presence of calcium. A recombinant AtCAMTA1 protein expressed in Sf9 insect cells bound to DNA-cellulose at low KCl and eluted at 200 mM KCl. Arabidopsis cell fractionation revealed that AtCAMTAs are localized in the nucleus. A yeast cell system was used to test the ability of chimeric fusion proteins, comprised of the DNA-binding domain of the bacterial LexA protein with different segments of AtCAMTA1, to activate transcription of a reporter gene. This revealed a distinct domain of AtCAMTA1 capable of activating transcription. Two cDNAs encoding human CAMTAs were also shown to activate transcription in yeast. We suggest that CAMTAs comprise a conserved family of transcription factors in a wide range of multicellular organisms. These transcription factors likely respond to calcium signalling by direct binding of calmodulin (Bouché et al., 2002, Journal of Biological Chemistry, in press).

4-21 Analysis of genes regulating plant perception of cytokinins

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The morphogenesis of plant organs is a directed process influenced by both genetic and environmental signals. Plant growth regulators (PGRs) are one link between environmental stimuli and the expression of genes. Cytokinins are known to be involved in a large range of important developmental processes, from embryogenesis to flowering. However, genes encoding proteins involved in the perception of cytokinin have proved particularly elusive, highlighting the importance of this PGR. A cytokinin binding protein (CBP) was isolated from cauliflower meristematic tissue, and genes encoding similar proteins have been identified in tomato, tobacco and Arabidopsis. These show sequence similarity to genes encoding the osmotin protein family. Initial expression analysis has shown high levels of mRNA accumulation in flower tissue. With further molecular and biochemical characterisation of these genes and proteins in tobacco and Arabidopsis we aim to elucidate their roles in cytokinin perception.

4-22 Arabidopsis *OLD1* defines a novel link between ethylene and leaf senescence

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The onset of leaf senescence is controlled by leaf age and ethylene can promote leaf senescence within a specific age window. We are interested in defining genes that regulate the onset of leaf senescence. By exploiting the interaction between leaf age and ethylene, we defined the senescence window in Arabidopsis. Subsequently a screen system was established and a range of *onset of leaf death* (*old*) mutants with early and late senescence phenotypes isolated. This poster describes the characterisation of *old1*. Both air-grown and ethylene-treated *old1* plants showed earlier leaf senescence, as envisaged by the accelerated visible yellowing, faster changes in chlorophyll content and ion leakage, and the expression profile of senescence-associated genes. In addition to senescence phenotypes, *old1* also exhibited a hypersensitive response to ethylene. Thus, the *old1* mutation generated alterations in two aspects: ethylene sensitivity and age-regulated leaf senescence. Double mutants were constructed between *old1* and mutants in the ethylene-signalling pathway, and initial analysis showed complex interactions between *OLD1* and ethylene-signalling genes. We present evidence to demonstrate that *OLD1* defines a novel link between ethylene and leaf senescence.

4-23 The *orc* mutant links sterols to cell polarity

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Correct polar auxin transport is necessary for patterning the Arabidopsis root meristem, which has been visualized by the auxin-responsive DR5::GUS reporter construct (Sabatini et al 1999). We isolated the *orc* mutant in a large scale EMS screen for mutants with a defective root meristem. Altered DR5::GUS distribution, absence of the quiescent center as well as cell polarity defects in columella cells and trichoblasts were observed in *orc* roots. Application of 2,4-D rescued trichoblast polarity defects and gave accumulation of DR5::GUS at low concentrations, which is consistent with defects in efflux. To understand the molecular basis of the *orc* phenotype, the *ORC* gene was cloned and found to encode Sterol Methyltransferase 1. *orc* has an increase in cholesterol and a decrease in sitosterol, both major membrane sterols. Immunolocalization of auxin carriers in *orc* showed disturbed localization of efflux carriers PIN 1 and PIN3, whereas AUX 1 is normally localized in *orc*. We postulate that, the *ORC* gene influences localization of specific proteins in the membrane to establish cell polarity and pattern.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P., and Scheres, B. 1999. An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. Cell 99: 463-472.

4-24 The homologous ABI5 and EEL transcription factors function antagonistically to fine-tune gene expression during late embryogenesis

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In Arabidopsis, the basic leucine zipper (bZIP) transcription factor ABI5 activates several late embryogenesis-abundant (LEA) genes, including *AtEm1* and *AtEm6*. However, the expression of many other seed maturation genes is independent of *ABI5*. We thus investigated the possibility that ABI5 homologues also participate in the regulation of gene expression during seed maturation. We identified a total of 13 *ABI5* -related genes in the Arabidopsis genomic sequence. RNA gel blot analysis showed that 7 of these genes are active during seed maturation and that they display distinct expression kinetics. We isolated and characterized two mutant alleles of one of these genes, *AtbZIP12/EEL*. Unlike *abi5*, the *eel* mutations did not inhibit the expression of any of the maturation marker genes that we monitored. On the contrary, the accumulation of the *AtEm1* and *AtEm6* mRNAs was enhanced in *eel* mutant seed as compared to wild-type seed. Gel mobility shift assays, combined with analysis of the genetic interactions among the *eel* and *abi5* mutations, indicate that ABI5 and EEL compete for the same binding sites within the *AtEm1* promoter. This study illustrates how two homologous transcription factors can play antagonistic roles to fine-tune gene expression.

4-25 *PASTICCINO* genes are repressors of cytokinin responses

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PASTICCINO genes, (*PAS1, PAS2* and *PAS3*), are involved in the control of cell division and differentiation during plant development. *Pasticcino* mutants have altered embryo, leaf and root development with finger-like cotyledons, short and thick hypocotyl and fused leaves. Tissue de-differentiation and ectopic cell division are observed in the cotyledons and the hypocotyl. Cytokinins but not auxins enhance cell proliferation leading to disorganized tumorous-like tissue. Differentiated cells of the *pas* mutants are more competent for cell division as illustrated by ectopic expression of cell division markers. Similarly, strong and enlarged expression pattern of a *KNAT* meristematic marker, is observed in *pas* mutants like in cytokinin treated wild type. Cytokinins induce carbohydrate accumulation (free sugars and starch) in wild type, which is also observed in *pas* mutants in absence of cytokinin treatment. Hypersensitivity of *pas* mutants to cytokinins is confirmed with the enhanced expression of the primary cytokinin response markers *ARR5* and *ARR6*. Deregulated expression of *ARR5* and *ARR6* were not associated with enhanced expression of their positive regulators, *ARR2* and *CKI-1* nor of the cytokinin regulated cyclin D, *CYCD3*. Contrary to cytokinin responses, which are increased, auxin responses are down regulated in *pas* mutants leading to probable amplification of the cytokinin phenotype.
Altogether our results suggest that *PAS* genes are involved in the hormonal control of cell division and differentiation by repressing cytokinin responses.

4-26 Signaling role of protein kinases in proteasome-associated SCF ubiquitin ligases

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Plant Snf1-related protein kinases (SnRKs) are conserved functional orthologs of the Snf1 (sucrose nonfermenting) and AMP-activated protein kinases (AMPKs) that play a central role in the regulation of glucose and stress signaling in yeast and animals, respectively. In different holoenzymes the regulatory domains of three Arabidopsis SnRK alpha subunits show a combinatorial interaction with two isoforms of AtSNF4 gamma subunits, which are produced by differential splicing, and two potential substrate targeting AKIN beta 1 and 2 subunits. Subunits of Arabidopsis SnRKs recruit various signaling proteins and thereby target the kinases to different regulatory complexes, such as the proteasome and spliceosome. SnRKs are found in stable complex with the ASK1/SKP1 subunit of SCF ubiquitin ligases that are recruited to the proteasome by interaction of SnRK and ASK1/SKP1 proteins with the alpha4/PAD1 subunit of proteasome catalytic cylinder. Phosphorylation by SnRKs may thus to regulate the SCF-mediated ubiquitination and subsequent proteolysis of important signaling factors. The activity of SnRKs is inhibited by a nuclear regulatory WD-protein, PRL1, which competes with the alpha4/PAD1 and ASK1/SKP1 proteins for binding to the regulatory domains of kinase catalytic subunits. PRL1 is a conserved spliceosomal protein, which appears to recruit SnRKs and the PAM1 protein methylase to their nuclear substrates, including some spliceosomal assembly factors. Pleiotropic effects of the *prl1* mutation, causing hypersensitivity to glucose, sucrose, auxin, ethylene, cytokinin and abscisic acid, suggest that SnRKs and their interacting partners are implicated in coordinate control of essential signaling pathways similarly to their homologs in other eukaryotic organisms.

4-27 A knock-out mutation in *Allene Oxide Synthase* in jasmonate biosynthesis results in male-sterility and defective wound signal transduction in Arabidopsis

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Recent studies on jasmonic acid (JA) biosynthetic mutants have shown that JAs play essential roles in pollen maturation and dehiscence and wound-induced defense against biotic attacks. To better understand the biosynthetic mechanisms of this essential plant hormone, we isolated an Arabidopsis knock-out mutant defective in the JA biosynthetic gene, *CYP74A*, *allene oxide synthase* (*AOS*) which catalyzes the dehydration of the hydroperoxide to an unstable allene oxide in the JA biosynthetic pathway. Endogenous JA levels, which increase 100-fold 1h after wounding in wildtype plants, do not increase after wounding in the *aos* mutant. In addition, the mutant showed severe male-sterility due to defects in anther development. The male-sterility of *cyp74A1* was completely rescued by exogenous application of MeJA and by complementation with constitutive expression of the AOS gene. RT-PCR analysis showed that the induction of transcripts for Arabidopsis *VSP2* and *LOX2*, previously shown to be inducible by wound and JA application in the wildtype, was absent in the aos mutant. In transgenic plants constitutively expressing *AOS*, wound-induced JA levels were 50-100% higher compared to wildtype plants. Taken together with JA deficiency in the aos mutant, our results show that AOS is critical for the biosynthesis of all biologically active jasmonates. Our results also suggest that *AOS* expression is rate limiting for JA levels in wounded plants, but that the AOS hydroperoxide substrate levels, controlled by upstream enzymes (lipoxygenase and phospholipase), determine JA levels in unwounded plants.

4-28 Auxin-regulated lateral root formation by *SLR/IAA14* and *SLP* (*SLR SUPPRESSOR*) genes

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To elucidate the molecular mechanisms of auxin-regulated lateral root formation, we are studying the *solitary-root* (*slr*) mutant in Arabidopsis, which has a gain-of-function mutation in IAA14, a member of Aux/IAA family (Fukaki et al., *Plant J*. 2002, 29, 153-168). The *slr* mutant has no lateral roots, few root hairs, and abnormal root and hypocotyl gravitropism. To identify genes that genetically interact with the *SLR/IAA14* gene in lateral root formation, we screened EMS-mutagenized *slr* M2 seedlings and identified at least three extragenic suppressor mutations for *slr*, that partially restore the *slr* phenotype. The *slp2* (*slr suppressor2*) is a single recessive mutation on the chromosome 2. The *slp2 slr* double mutant produces lateral roots but still has few root hairs and abnormal gravitropism, indicating that the *slp2* mutation specifically suppresses the *slr* defect in lateral root formation. The other two mutations, *slp1* and *slp3*, also suppress the lack of lateral roots in *slr*. These results indicate that the *SLP1*, *SLP2*, and *SLP3* genes genetically interact with *SLR/IAA14* in lateral root formation. Progress on phenotypic characterization of these slp mutants and the map-base cloning of *SLP2* gene will be presented.

4-29 Analysis of *cis* regulatory element involved in expression of *AtGA3ox1*, encoding gibberellin 3-oxidase

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From germination to flowering, gibberellin (GA) plays important roles in plants' development and response to some exogenous stimuli. To elucidate the mode of its action, the regulation of its biosynthesis and signal transduction must be understood. All enzymes consisting GA biosynthesis pathway are already identified in *A.thaliana*, and their regulation is now under investigation. Among them, GA 3-oxidase catalyzes the final step of production of active gibberellins, and seems to be a key enzyme in GA biosynthesis. In *A.thaliana*, there are four putative GA 3-oxidase genes. We analyzed expression patterns of these four genes and found each gene shows specific expression pattern. Especially, *AtGA3ox1* is supposed to play as a major GA 3-oxidase in A.thaliana, because of its high and general expression. *AtGA3ox1* is also known to be under the negative feedback regulation in response to endogenous active gibberellins at transcriptional level. Because this regulation is disrupted in GA signaling mutants, it may link GA biosynthesis and GA signal transduction, and contribute to GA homeostasis. To unravel the mechanism underlying the negative feedback regulation of *AtGA3ox1*, we proceeded to identify transcription factors directly participating in it. We first made transgenic plants containing *GUS* reporter fusions with *AtGA3ox1* promoters and deletion analysis were performed. In result, the cis-region responsible to the regulation was shortened to 200 bp, and this region is able to confer the negative feedback responsibility to a minimal promoter. Now is more detailed analysis underway.

4-30 The *LATE ANTHER DEHISCENCE* gene: A link between anther opening and auxin signalling?

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The I-element tagged *lad* (*late anther dehiscence*) mutant of Arabidopsis is impaired in proper anther dehiscence, leading to partial male sterility. The only defect found in mutant plants, compared to Landsberg *erecta* is delayed degradation of the cell walls of anther septum cells. The gene encodes a protein with homology to MYC proteins, containing an acidic domain and a basic helix-loop-helix domain, indicating a role in transcriptional regulation. Promoter-GUS fusions and mRNA in situ hybridisations show the gene to be expressed in anthers, but also in the pistil. Overexpression of the *LAD* gene unexpectedly caused a pin-like phenotype indicating impaired polar auxin transport. However, auxin transport was measured in stem pieces and found unaltered compared to Columbia wildtype. The role of the *LAD* gene and auxin signalling in the process of anther dehiscence will be discussed.

4-31 The *AtRHA1* gene of Arabidopsis: Structure and possible functions

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The mutant *rha1* shows in the roots reduced gravitropic response, reduced slanting toward the right-hand, and increased resistance to the auxinic hormones, their inhibitors, and ethylene. Taking advantage of a T-DNA tag inserted in AtRHA1, the gene was cloned through the TAIL-PCR technique. The gene appears to be a new heat shock factor (HSF), made up of three EXONS, two of which translated, and two INTRONS. It maps on chromosome 5, close and above the RFLP marker mi61. RT-PCR confirmed that the gene is expressed in the wild-type, but not in the mutant. RHA1 shows notable homology in the DNA binding motif, at the level of the aminoacidic sequences, with other HSFs from plants (Arabidopsis, *L. peruvianum*, maize), Coenorhabdites, Drosophila, mouse, yeast, and humans. It belongs to the B group of these proteins, but is lacking of the terminal hydrophobic repeat HR-C, generally present in other HSFs. We hypothesize that RHA1, apart its function in the activation of HSPs, could also be involved in the transduction of signals coming from gravity and auxin. Possibly, as shown for the HSF2 from humans (Hong and Sargent 1999, J. Biol. Chem. 274, 12967- 70), it could be involved in the regulation of the PP2A phosphatase, which has been shown to regulate the trasport of auxin in Arabidopsis roots (Garbers et al. 1996 EMBO J. 15, 2115-24).

4-32 The Arabidopsis *URO* gene is involved in the establishment of apical dominance and the fiber induction

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It is usually thought that plant hormone auxin plays an important role in the establishment of apical dominance as well as the fiber induction in vascular tissues through its polar flowing. In order to improve our understanding of auxin regulation in plant development, we screened for mutant plants that are loss of plant apical dominance. We reported previously the identification and characterization of a semidominant mutant *uro* and genetic mapping of the *URO* gene (Sun et al., 2000). Here we report more detailed phenotypic analyses of *uro* mutant, and propose that the *URO* gene may regulate auxin polar transportation from SAM to the other part of a plant. Plant homozygous for *uro* is dwarfish with a very short primary inflorescence stem, while the axillary inflorescence stems are predominant. Interestingly, some axillary inflorescences are replaced by a cup-like leaf. In addition, all *uro* stems are very soft, and cross sections showed that secondary xylem and inter fascicular fibers in the inflorescence stems were missing. In order to provide a direct link between *URO* function and anxin regulation, we constructed *uro* and *pin1* double mutant and analyzed *uro pin1* phenotypes. The *uro pin1* plant, like *uro* single mutant, also shows the loss of apical dominance. Unlike *pin1* and *uro* single mutants, the *uro pin1* inflorescence contains numerous flowers, mimicking the results that the *pin1* inflorescence was treated with auxin (Reinhardt et al.; 2000). It is possible that *uro pin1* inflorescence accumulates a high concentration of auxin, and the mutation of *URO* causes aberrant auxin transportation out of the place where it is produced.

4-33 Cross-talk in signal transduction

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To understand the interaction of light and hormones, several mutants of Arabidopsis have been isolated based upon the phenotype of young, dark-grown seedlings. One of these mutants, *cah1* (*c* ytokinin *a*uxin *h*omeostasis), displays abnormalities reminiscent of altered auxin-cytokinin ratio. The auxin-related phenotype of the mutant includes short hypocotyl, open apical hook, agravitropism, inhibited shoot growth, curled leaves and short roots with increased adventitious branching. The young mutant seedlings show enhanced GUS expression under the influence of auxin inducible *TCH4* and *SAUR* gene promoters (as analysed by GUS staining). Although the mutant shows normal sensitivity towards auxin as well as auxin polar transport inhibitor, TIBA, but it shows an overall resistance towards cytokinin in hypocotyl/root growth inhibition assays. The other cytokinin-related features include reduced regeneration in tissue cultures and decreased sensitivity to cytokinin for anthocyanin induction. Since *IBC7* (*i*nduced *b*y *c*ytokinin) gene is normally induced both in the WT and the mutant, lesion is probably in the cytokinin signal transduction pathway downstream to *IBC7*. It thus appears that the auxin-exaggerated phenotype displayed by the mutant is due to altered auxin-cytokinin homeostasis. Besides these features, this mutant also shows partial photomorphogenesis in dark and sugar hypersensitivity. The genetic analysis has revealed that the mutant *cah1* is non-allelic to *rooty* and *det3* , the two mutants that resemle *cah1* phenotypically to a certain extent. What precisely is the function of CAH1 will be revealed only by its molecular cloning but these studies do indeed suggest that it plays an important role in multiple signaling pathways.

4-34 *PIN* genes and root development

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In Arabidopsis, polar auxin transport is mediated by combined activities of influx and efflux carriers. Evidence has accumulated that the Arabidopsis PIN gene family encodes putatives efflux carriers. Several members of these family were recently described and their expression is restricted to specific but overlapping regions. Application of auxin transport inhibitors (NPA) in Arabidopsis roots alters auxin distribution and leads to severe developmental defects (Sabatini et al., 1999), indicating that auxin efflux is crucial for root patterning. However, the Atpin mutants described until now have subtle root phenotypes indicating that the abscence of one member is not sufficient to dramatically alter the root pattern.
To test the full extent of PIN gene function in roots, we analyzed combinations of multiple pin mutants. When double mutants were analyzed, only additive phenotype were observed in all combinations. However some triple mutants, showed enhanced defects in the meristem, which became more severe in quadruple mutant combinations. Through our approach, we are unravelling new redundantly encoded functions of the PIN gene family in root development.

4-35 SCARECROW, polar auxin transport and quiescent center specification in the Arabidopsis root

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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. In the Arabidopsis root meristem, the stem cells surround four mitotically inactive cells, the quiescent center (QC). Laser ablations have indicated that contact with the QC is required to maintain stem cells status (van den Berg, 1995, *Nature*).
The SCARECROW (SCR) gene is expressed in the QC precursor cells from early embryogenesis onward (Malamy & Benfey 1997 *Development*). We show that scr root mutants display lack of QC markers and reduced stem cell activity.
Here we show that the SCR protein acts cell autonomously to establish QC identity and that its expression in the QC is sufficient for non-autonomous maintenance of the surrounding stem cell. What selects only four cells of the SCR expression domain as QC cells? We have previously shown that polar auxin transport provides a spatial cue for QC formation in the post-embryonic root. In the *orc* mutant QC markers are absent while SCR is transcribed suggesting that ORC defines a SCR-independent input for QC specification. Interestingly *orc* mutant causes a strong reduction in polar auxin transport.
Taken together these data suggest that polar auxin transport selects a subset of SCR expressing cells to define the QC and, as a result, the root stem cell population.

4-36 Control of ethylene synthesis by expression of ACC deaminase from *Schizosaccharomyces pombe* in transgenic *Arabidopsis thaliana* plants

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Synthesis of the phytohormone ethylene is believed to be essential for many plant developmental processes. The control of ripening in climacteric fruits and vegetables is among the best characterized of these processes. One approach to reduce ethylene synthesis in plants is to modify the metabolism of its immediate precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). The gene encoding ACC deaminase from *Schizosaccharomyces pombe* was introduced into the pBINPLUS vector under the control of the CaMV 35S promoter. This construct was used in a *Agrobacterium tumefaciens*-mediated transformation and Arabidopsis plants were selected by resistance to kanamycin. Expression of the ACC deaminase gene in the transformed plants was proved by RT-PCR. The reduction in ethylene synthesis in transgenic plants caused an apparent vegetative phenotypic abnormalities. Flowers and fruits from these plants exhibited significant delays in ripening, and siliques remained firm for at least 6 weeks longer than in the nontransgenic control plants. These results indicated that ACC deaminase from *S. Pombe* is useful for examining the role of ethylene in many developmental and stress-related processes in plants. Additionaly could give a powerful tool for extending the vase life of ornamental plants and shelf life of fruits and vegetables whose ripening is mediated by ethylene.

4-37 Unfolded protein response signal transduction in Arabidopsis

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The unfolded protein response (UPR) is a transcriptional and translational intracellular signaling pathway activated by the accumulation of unfolded proteins in the lumen of the endoplasmic reticulum (ER). The primary UPR targets are molecular ER chaperones and folding enzymes; induction of these proteins augments the capacity of the protein folding system, leading to homeostasis of the ER. This response seems to be conserved among different organisms from yeast to mammals.
We have previously described that Arabidopsis has a normal UPR process and the characterization of Arabidopsis AtIre1-1 and AtIre1-2 proteins, whose homologs are involved in UPR signaling. Both AtIre1-1 and AtIre1-2 proteins share common features with their yeast and mammalian homologs, but if they are involved in the UPR of plant cells still remains to be demonstrated. The AtIre1 mRNA levels in plants are very low and may be autoregulated, possibly by its own ribonuclease domain, preventing any overexpression of the protein and, therefore, a constitutive UPR. Single KO mutants for cytoplasmatic truncated AtIre1 forms are phenotipically identical to wild type plants. Moreover, induction of the BiP mRNA is also detected in those mutants when they are treated with tunicamycin. We carried out expression profiles in Arabidopsis plants treated for 2 and 5 hours with either tunicamycin or dithiothreitiol. The results show that most of the genes that were clearly upregulated fell into categories that related them clearly to the function of the ER secretory system, vacuoles, or proteolysis. A group of upregulated genes did not fall into these categories.

4-38 A yeast two-hybrid interaction screen for upstream partners of AtSERK1

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The *AtSERK1* gene encodes a leucine rich-repeat (LRR) transmembrane receptor-like kinase that enhances the embryogenic competence of tissue in culture. The gene is expressed in ovules and in the entire female gametophyte before and after fertilization (Hecht *et al.*, 2001). To identify other components of the membrane receptor complex we performed a yeast two-hybrid screen using the AtSERK1 extracellular domain as bait and identified several interactors. One of them is a small 220aa Cys-rich protein that shows homology to the C-terminal extension of a special class of Arabidopsis ß-1, 3-endoglucanases (Hird *et al.,* 1993). This protein belongs to a small gene family of four members in the Arabidopsis genome. The presence of an EGF Cys-reach domain was identified by SMART program. Deletion experiments in yeast have shown that the protein possibly interacts with the SPP rich domain of the AtSERK1 protein and that the interaction is specific, as the protein does not interact with the close homologue of AtSERK1, AtSERK2. RT-PCR experiments have shown that the gene is expressed in all plant tissues. CFP/YFP fusions were used to localize the protein in cowpeas protoplast where a signal was seen on the plasma membrane and a little expression in the cytoplasm. The Cys-rich protein also co-localizes with the GFP fused AtSERK1 protein. FRET and protein pull-down experiment are carried out to further confirm the specificity of interaction *in vivo* and *in planta*.

Hecht *et al.,* (2001) Plant Physiology 127: 803-816; Hird *et al.,* (1993) The Plant Journal 4(6): 1023-1033

4-39 COI1 links jasmonate signalling and fertility to the ubiquitination complex in Arabidopsis

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coi1 mutant is insensitive to MeJa and coronatine-induced root growth inhibition, is male sterile and does not express MeJa regulated genes including *thionin 2.1* and *VSP*. *COI1*, has been mapped by positional cloning and it encodes an F box protein with 16 leucine rich repeats (LRRs) (Xie *et al.* , 1998, Science, 280, 1091-1094). F box proteins, together with Skp1 proteins have been identified as components of the SCF complex. We demonstrate that COI1 interacts with components of the SCF complex and with a repressor of transcription, in yeast and *in planta*.
Three classes of clones (CIPs) were isolated in a yeast-two hybrid screen, and co-immunoprecipitation experiments with epitope-tagged COI1 confirmed the interaction in Arabidopsis plants and cell cultures with SKP1 like proteins, cullin and a histone deacetylase. Deletion analysis in yeast revealed that COI1 F-box and LRR are differentially required for the interaction. We also demonstrate the specificity of the identified interactions for COI1 over TIR1. Our data suggest that COI1 forms an SCFCOI1 complex *in vivo*. COI1 is therefore expected to form a functional E3-type ubiquitin ligase in plants recruiting transcriptional repressors of jasmonate responsive genes for ubiquitination. We also compared the evolution of male meiosis leading to production of pollen grains in *coi1*-1 and *ask1* -1 and we will show phenotypic analysis of a CIP.
This research is supported by BBSRC.

4-40 The control of ethylene biosynthesis under oxidative stress

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Ozone induces oxidative stress leading to programmed cell death in sensitive plants. Ozone-induced cell death resembles pathogen-induced hypersensitive response (HR). Similar to the HR, ozone-induced cell death is an active process requiring signalling and changes in gene expression. We use ozone as a model for the activation of cell death by reactive oxygen species. Utilizing a genetic approach we have isolated several unique oxidative stress sensitive mutants in *Arabidopsis thaliana*. The co-dominant mutant *rcd1* (*radical-induced cell death 1*) exhibits accumulation of cellular superoxide and transient spreading lesions when exposed to ozone. We have previously shown that ethylene is required for ozone-induced cell death, however the factors regulating stress ethylene biosynthesis remain poorly understood. Ethylene is synthesized via the precursor 1-aminocyclopropane-1-carboxylic acid (ACC), where the formation of ACC by ACC synthase (ACS) represents the rate-limiting step. From the six studied ACC synthase genes, *At-ACS6* alone is responsible for ozone-induced ethylene synthesis. We aim to understand the regulation of ethylene synthesis and to study the kinetics and spatial localization of ethylene production with respect to cell death and radical formation. To this end, transgenic Arabidopsis *rcd1* and Col-0 lines harboring an *At-ACS6* promoter-GFP reporter construct and *rcd1* plants with an *At-ACS6* antisense construct were created. Studies with these transgenic lines will help to elucidate the role of ethylene in oxidative stress. Crossing these lines into various mutant backgrounds will allow genetic dissection of signals controlling stress ethylene biosynthesis.

4-41 Ankyrin protein kinases: A novel type of plant kinase genes

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Msapk1, for *Medicago sativa* ankyrin protein kinase 1, codes for a novel type of plant protein kinase containing an N-terminal region with an ankyrin repeat fused to the kinase domain. Several genes coding for related ankyrin-protein-kinases (APKs) were found in plants and animals. Animal related kinases are likely involved in connecting the extracellular matrix and the cytoskeleton during cell adhesion. Two of the three closest homologs from *Arabidopsis thaliana* (*Atapk1* and *Atapk2*), were shown to express differentially using gene-specific RT-PCR. *Atapk1* was preferentially expressed in roots whereas *Atapk2* showed stronger expression in stems and leaves. Comparison of *M. truncatula* and Arabidopsis gene regions suggests that the Arabidopsis sequences may have duplicated after speciation. The *Atapk* genes also share several neighbouring genes between them. Exon/intron distribution was the same for all expressed genes of both species. A T-DNA insertion mutant in *Atapk1* was identified and Arabidopsis plants were prepared in order to express MsAPK1 or a dominant negative DN form. None of these plants showed an evident phenotype, however expression of another MsAPK1 mutant form affected Arabidopsis growth. Biochemical approaches are being done to characterise APK activities in plants in order to link their function to cytoskeletal changes in plant development.

4-42 The cloning of RCD1, a multifunctional transducer of hormone signals

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We have introduced the radical hypersensitive *radical-induced cell death 1 (rcd1)* mutant as a model for studying the activation of HR-like programmed cell death. *rcd1* has yielded insights into the regulation of cell death by ethylene and jasmonate (Plant Cell 12: 1849). Recent results highlight the role of RCD1 in hormone signaling. Here we focus on the hormone-related phenotypes and the positional cloning of *rcd1*. Several visible phenotypes are associated with the mutation: *rcd1* rosettes are smaller with short petioles and more erect, serrated, and waved leaves. Primary root length is shorter while secondary roots are longer. The onset of flowering and senescence are premature in rcd1. An increased number of secondary shoots and decreased primary shoot length indicate reduced apical dominace. *rcd1* plants show deficiencies in multiple hormone-signaling pathways. Macroarray, RNA gel blot, and other experimental data indicate that jasmonate, ethylene, and abscisic acid (ABA) signaling are all altered in rcd1. The most pronounced deficiency is in ABA-signaling as several marker genes are even less ABA-responsive in *rcd1* than in *abi1*. *rcd1* has also elevated basal ethylene evolution, which, when exposed to superoxide-forming conditions increases highly. Additionally, *rcd1* has features of jasmonate insensitivity when analyzed with marker gene (*pdf1.2*) expression. The *rcd1* locus has been mapped to a region containing seven genes. A mutation in one of these genes disrupts a conserved splice site and rtPCR analysis verifies a mispliced intron, which results in premature stop codon. The structure and putative role of RCD1 in hormonal interactions is discussed.

4-43 ABI4 and sugar induced signalling

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Carbohydrates are important for plants as energy source and as structural and storage components. Next to that sugars have a signalling function and to understand this mechanism several laboratories set up screens to isolate sugar insensitive mutants. In our laboratory a mutant screen was conducted using a transgenic Arabidopsis line, harbouring a PC promoter fused to luciferase (Pc-Luc line) (Dijkwel *et al*., 1997). Three days old seedlings show, a developmentally regulated, transient increase of PC expression which can be suppressed by adding 3% sucrose in the media. We were able to identify 18, so called *sun* (for *s*ucrose *un*coupled) mutants, which do not show this sucrose induced down regulation of PC expression in young, dark grown seedlings. The defective gene in the *sun6* mutant has been identified and turned out to be a new ABI4 allele (ABA Insensitive4) (Huijser *et al*., 2000), previously isolated by Finkelstein *et al*. (1998). Also other research groups identified new ABI4 and ABA2 alleles in their sugar insensitive screens that underlines the importance of an intact ABA signal transduction chain for proper sugar responses. Both ABA and sugars influence gene expression and affect plant processes throughout plant life. Therefore we characterized *sun6*/*abi4* with respect to germination, early seedling development, growth and flowering.

References:
Dijkwel *et al*. (1997) Plant Cell 9, 583-595
Huijser *et al*. (2000) Plant Journal 23: 577-585
Finkelstein *et al*. (1998) Plant Cell 10,1043-1054

4-44 Molecular/genetic analysis of brassinosteroid function

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Brassinosteroids (BRs) comprise a group of polyhydroxysteroids and represent a recently identified class of phytohormones showing structural similarities to steroid hormones from mammals and arthropods. They occur ubiquitously in plants to control various developmental and metabolic processes, and are integrated into a complex regulatory network, which includes several other plant hormones. A number of BR-biosynthetic mutants have hither been isolated. They include for example the BR-deficient mutant dwarfs cbb1/dwf1/dim and cbb3/cpd as well as the BR-insensitive cbb2/bri.

In order to gain insight into the molecular mechanisms underlying BR action BR-regulated genes have been isolated by means of subtractive suppression cDNA cloning. Among approximately 200 clones checked, about 20 proved to be differentially expressed upon BR-treatment including cDNAs encoding OPR3, CAB, Rubisco, RSZp22/21 protein, Retrovirus-like sequences, chloroplast-proteins and several proteins of unknown function.

The BR-inducible "*SL55*" gene thus identified encodes putative transcription factor and was chosen for detailed analysis. "*SL55*" gene is unique in Arabidopsis. It is expressed in all organs and the protein is supposedly located to the nucleus. Analysis of a putative loss of function T-DNA insertion mutant revealed further information on the function of this gene.

4-45 *STY1* and *STY2*, two genes with putative roles in auxin regulated processes

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The development of the Arabidopsis gynoecium is a complex process involving a large number of genes. Auxin has been suggested to act as a morphogen in this process directing both patterning and vascular development. We have identified two related genes, *STYLISH1* (*STY1*) and *STYLISH2* (*STY2*), with roles in style and stigma formation as well as in vascular development in the gynoecium. An insertion mutation in *STY1* causes aberrant style and stigma development together with a slight reduction in stylar xylem proliferation and basialisation of medial vein bifurcation in the gynoecium. *sty2* mutant plants exhibit no visible phenotype but enhances the *sty1* phenotype in *sty1sty2* double mutant plants.
As the defects in style and stigma formation in the *sty1sty2* mutants may be connected to changes in auxin sensitivity or accumulation we crossed *sty1sty2* to the weak PINOID mutant *pid-8*. *PID* has been suggested to be an enhancer of polar auxin transport. Preliminary results suggest that *sty1sty2* enhances the *pid* phenotype implying that *STY1* and *STY2* may be needed for *PID* function.
Over expression of *STY1*, *STY2* or the closely related gene *SHI* (*SHORT INTERNODES*) has pleiotropic effects on plant development including dwarfism, dark green narrow leaves, reduced number of lateral roots, malformed siliques, and reduced apical dominance. The reduced number of lateral roots in plants over expressing *SHI* is completely restored by the external application of low amounts of auxin. This implies that auxin amounts are sub optimal for lateral root formation in these plants.

4-46 The role of AXR3/IAA17 in root hair formation

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AXR3/IAA17 belongs to the *AUX/IAA* family of auxin induced transcriptional regulators. They encode low abundance, nuclear proteins with a very short half-life. The *AXR3* gene has been previously characterised by the introduction of two semi-dominant mutations (axr3-1 and *axr3-3*) which affect many auxin-regulated evelopmental processes. The mutations lie in domain II of the protein and confer a higher degree of stability.One of the major phenotypic effects of *axr3-1* is that the root does not produce any root hairs. *AXR3* is believed to act as a suppresser of root hair formation in the elongation zone. In the root hair formation zone, it is probable that either reduced expression of the *AXR3* gene or increased instability of the AXR3 protein allows the formation of hairs. In the *axr3*-1 mutant the increased stability of the protein may allow it to persist into the zone of root hair formation, thus blocking the development of root hairs, or alternatively affecting the expression of itself and other *Aux/IAAs*, resulting in the continuation of its expression in the zone of root hair formation. This has been tested using the GAL-4 transactivation system to target misexpression of *axr3-1* to specific cell types. Root hair development has been characterised, using molecular and genetic techniques, in a range of *AXR3* mutant backgrounds, alongside mutants of another *Aux/IAA, SHY2/IAA3. SHY2* is of particular interest as the gain-of-function mutant, *shy2-2*, has an opposite root hair phenotype to *axr3-1*.

4-47 Transgenic plants expressing RNAi for RUB1 exhibit defects in multiple hormonal signaling pathways

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Rub is a 76-amino acid ubiquitin-like protein similar to ubiquitin in mechanism of conjugation. The lab has previously identified three genes encoding Rub proteins in *Arabidopsis thaliana*. RUB1 and RUB2 differ by only 1 amino acid, but RUB3 has 16 amino acid substitutions from RUB1. In vitro work using the RUB1 ortholog in mammals, NEDD8, has confirmed its function in a ubiquitin ligase complex, called the SCF. RUB1 conjugation in Arabidopsis has been linked with auxin signaling. *AXR1* (Auxin Resistance 1) encodes the N-terminal half of an enzyme with similarity to the ubiquitin activating enzyme and activates RUB1 in vitro. In order to determine the role RUB1 plays in vivo, we are analyzing the effects of reducing RUB1 protein level in Arabidopsis using a PTGS (Post-Transcriptional Gene Silencing) technique. We have produced several stable T3 RUB1 PTGS lines. They exhibit an extremely dwarfed phenotype, greater than an axr1 null mutant, and show increased auxin resistance over wild-type lines. Mutant seedlings, grown in the dark, have a characteristic triple response phenotype, associated with a response to high ethylene levels. This phenotype is suppressed when seedlings are grown on either AgNO3 or AVG, supporting the hypothesis that they are overproducing ethylene. We propose that RUB1 conjugation is not solely dependent on AXR1 owing to the severity of the RUB1 PTGS phenotype relative to the *axr1-12* phenotype. This is further supported by the observation that CUL1, a proven RUB1 conjugate, maintains a slower migrating form in the absence of AXR1.

4-48 Characterization of the *Brassica* self-incompatiblity protein, ARC1, and related Arabidopsis proteins: A family of U-box proteins with a role in the ubiquitination/proteasomal protein degradation pathway

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Arm Repeat Containing 1 (ARC1) is a novel protein, which has been shown to be involved in *Brassica* self-incompatibility. The amino acid sequence of ARC1 is predicted to contain a leucine zipper, coiled coil, U-box and seven arm repeats. ARC1 also contains a functional nuclear localization signal and two nuclear export signals, which ensure a predominantly cytosolic localization for ARC1. The U-box has recently been implicated ubiquitination/proteasomal protein degradation and a growing number of U-box containing proteins have been shown to posses E3 ubiquitin ligase activity, thus defining a new class of E3 ligases. Results from recent experiments including transient protein expression studies in tobacco BY2 cells have indicated that ARC1 may function within the ubiquitin/proteasomal pathway as an E3 ubiquitin ligase. We have begun to characterize a large family of predicted genes in Arabidopsis that show sequence identity to ARC1. Searches using various Arabidopsis databases with the ARC1 amino acid sequence have revealed more than 40 putative homologues. All the homologues examined contain a U-box followed by an arm repeat region of differing lengths. Comparison of predicted amino acid sequences between ARC1 and the Arabidopsis ARC1 family revealed highest levels of sequence identity in the U-box followed by the arm repeats. Northern blot analysis using representative members of the predicted ARC1 family have shown that these genes are expressed at various levels in stem, root, leaf, buds and root. Based on our recent findings we may have identified a new family of E3 ubiquitin ligases in Arabidopsis.

4-49 AtTPC1, a calcium permeable channel in Arabidopsis

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Calcium is a ubiquitous second messenger in plants, encoding specific responses to a wide range of biotic and abiotic stimuli. However, despite the numerous types of calcium-permeable channels characterised at an electrophysiological level, their molecular characterisation has long been awaited. Here we report the characterisation of AtTPC1 (Arabidopsis Two Pore Channel, At4g03560), a putative calcium channel.
AtTPC1 shows similarities with L-type voltage-gated animal calcium channels and comprises two repeats of six transmembrane domains. Using a yeast mutant deficient in calcium uptake, we showed that the expression of AtTPC1 partially complements the phenotype of this mutant and that the activity of the TPC1 protein was regulated by hydrogen peroxide. In yeast, the targeting of the TPC1 protein was mainly directed at endomembranes, which could explain the partial complementation. TPC1 failed to complement phenotypes of yeast K+ and Na+ transport mutants, suggesting a specificity for Ca2+ over monovalent cations.
Using promoter fusions and northern blotting, we showed that the expression of AtTPC1 in planta was ubiquitous and was regulated, to a certain extent, by external calcium concentrations. GFP fusion showed that TPC1 is mainly targeted to the plasma membrane in Arabidopsis. In order to assess the role of AtTPC1 in generating Ca2+ signals in Arabidopsis, plants overexpressing the protein were generated in lines expressing the Ca2+ reporter protein aequorin. The overexpression of TPC1 leads, to increased cold-induced cytosolic calcium spikes compared to control aequorin-expressing plants.

4-50 Guard cell abscisic acid signaling involves sphingosine-1-phosphate and heterotrimeric G proteins

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Stomata form pores on leaf surfaces that regulate uptake of carbon dioxide for photosynthesis and loss of water vapor during transpiration. The plant hormone abscisic acid (ABA) plays an essential role in adaptive stress responses by regulating turgor changes in the guard cell pair surrounding the stomatal pore. Recently, sphingosine-1-phosphate (S1P) was shown to be a novel calcium-mobilizing compound involved in drought/ABA regulation of stomatal closure in *Commelina communis* (Ng et al., 2001, Nature, 410: 596-599). Nevertheless, little is known about the cellular mechanisms regulating S1P action in plant cells. Here, we use leaf and guard cell extracts from *Arabidopsis thaliana*, to show that ABA activates sphingosine kinase *in vitro*. *N,N*-dimethylsphingosine, an inhibitor of sphingosine kinase in animal cells, significantly attenuates ABA inhibition of stomatal opening and promotion of stomatal closure, concomitant with changes in the activities of guard cell inward K+ channels and slow anion channels. Responses of stomatal apertures and guard cell ion channels to S1P are abolished in T-DNA knockout lines of the sole prototypical heterotrimeric G protein alpha subunit gene, *GPA1*. Our results implicate G proteins as downstream elements of the S1P signaling pathway mediating ABA regulation of stomatal function, and suggest that the interplay between S1P and heterotrimeric G proteins represents an evolutionary conserved signaling mechanism in eukaryotes.

4-51 Molecular characterization of the last subunit (CSN2) of the COP9 signalosome and overall analysis of the whole complex

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The COP9 signalosome (CSN) is an evolutionary conserved protein complex, which, through its ability to regulate ubiquitin-proteasome mediated protein degradation, controls many aspects of development. Arabidopsis CSN mutants belong to the class of the *cop/det/fus* mutants, displaying a constitutive photomorphogenic seedling phenotype and lethality before reaching adulthood. We report here the cloning and characterization of the subunit 2 of Arabidopsis CSN, the last uncharacterized subunit. Based on the peptide sequences obtained from the purification of cauliflower CSN, we have isolated the full-length cDNA encoding Arabidopsis CSN2. The AtCSN2 gene corresponds to the previously defined *FUS12* locus and AtCSN2 only exists in CSN. In addition, AtCSN2 interacts with AtCSN1 in the two-hybrid system, further confirming that AtCSN2 is an integral component of CSN. We have previously reported that AtCSN2 is capable of interacting with AtCUL1, the cullin subunit of the ubiquitin ligase SCFTIR1. Here, we show that AtCSN2 interacts not only with AtCUL1 but also with AtCUL3, implying that CSN is able to regulate the activity of several cullin-based E3 ligases through highly conserved interactions. The availability of the complete set of Arabidopsis and human genes encoding all eight CSN subunits allowed us to compare the phylogenetic relationship of CSN subunits together with subunits from the lid subcomplex of the proteasome. In addition, we will also present our progress toward the mapping of CSN subunit topology, based on an exhaustive interaction analysis among all CSN subunits.

4-52 SA-inducible CK2 activity may play a role in controlling the sub-cellular localization of TGA/OBF in Arabidopsis

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TGA/OBF proteins are a group of transcription factors, some of which have been shown to interact with NPR1, a key regulator of systemic acquired resistance. In the present study we have investigated whether TGA/OBF activity is regulated via phosphorylation. We found that some TGA proteins are phosphorylated both *in vitro* and *in vivo* by a CK2-like activity. TGA2 was chosen for the further analysis. This phosphorylation activity can also be induced by salicylic acid (SA), an important signal molecule for plant disease resistance. Induction of the TGA2 phosphorylation by SA suggests that this event may have a regulatory function in determining its interaction with NPR1 and/or other downstream signaling components. Therefore, the phosphorylation sites on TGA2 were mapped and then replaced with amino acids that either prevented phosphorylation of TGA2 or mimicked a constitutive phosphorylated amino acid. Wild type and altered forms of TGA2 were fused with GFP and transformed into Arabidopsis. Confocal microscopy with these transgenic lines revealed that alterations of the phosphorylation state of TGA2 may lead to changes in sub-cellular localization of TGA2 between the nucleus and cytosol.

4-53 Molecular cloning and functional characterization of a gene coding for a putative receptor-like protein kinase with a leucine-rich repeat, *IRK*, expressed in inflorescence and root apices from Arabidopsis

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We isolated a novel receptor-like kinase with leucine-rich repeat (LRR) by a differential screening for genes that were specifically expressed in inflorescence shoot apices of Arabidopsis, and named it *IRK* (*inflorescence and root apices receptor-like kinase*) after its expression pattern and structural features. The kinase domain expressed in *E. coli* showed an autophosphorylation activity *in vitro*, indicating that *IRK* encodes an active protein kinase. The IRK protein fused to green fluorescence protein was targeted to plasma membrane, indicating that IRK is a membrane-localized receptor kinase. *IRK* was expressed in shoot apices, root tips and developing flowers. The T-DNA tagged *irk* mutant did not show any visible phenotype, probably due to a unctional redundancy among receptor-like kinases encoded in the genome. To identify the downstream component of signaling mediated by *IRK*, we isolated a gene for target protein that interacts with the kinase domain of IRK by using yeast two-hybrid screening. The product of the candidate gene did not show any significant similarity to known proteins. The mutant for the gene by RNA interference showed decreased apical dominance, dwarfism, and reduction in cell number in stem tissue.

4-54 Isolation of Arabidopsis mutants affect on the abscisic acid sensitivity using an abscisic acid analog

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Abscisic acid (ABA) is involved in many physiological activities of plants, including the regulation of seed dormancy and responses to abiotic environmental stresses. Despite of its important function, we are still far from the fully-understand of the ABA signal transduction pathway. In order to identify the components involved in the ABA signaling pathway, we conducted a screen searching for mutants that have an altered ABA sensitivity using an ABA analog, PBI-51 (ARI), which acts as an ABA binding inhibitor in Brassica (Wilen et al., Plant Physiol., 101, 469-476, 1993). We isolated ABA-inhibitor-responsive (*air*) mutants, seven lines from wild-type Col back ground and several mutant candidates from Col aba2 back ground, that cannot germinate in the presence of 20 uM ARI, a concentration that allowed the germination of parental strain seeds. Since all the air mutants less germinates in the presence of 0.3 uM of ABA, these mutants are thought to be more or less ABA hypersensitive. Currently we are trying to characterize those mutants and map the mutated genes.

4-55 Characterization of the lipoxygenase gene family of Arabidopsis

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Lipoxygenases (LOXs) are ubiquitous enzymes in eukaryotes. They belong to the family of dioxygenases and catalyze the hydroperoxidation of polyunsaturated fatty acids, containing a (1*Z*, 4*Z*)-pentadiene double bound system. The resulting hydroperoxides serve as substrates for at least seven different enzyme families to produce physiological important compounds, like jasmonic acid, traumatic acid, volatile aldehydes, fatty acid divinyl ethers, and ketol fatty acids. Evidence is accumulating, that LOXs are involved in plants growth and development, wound response and pathogen resistance. However, the analysis of possible physiological functions in plants is complicated, because of the existence of multiple isoforms within one organism having redundant biochemical and therefore presumably overlapping physiological properties.
Since the genome of the model plant *Arabidopsis thaliana* is sequenced the absolute number of six LOX genes could be determined. In order to analyze all LOX isoforms we cloned all of them from different cDNA libraries by PCR based on sequences of the Arabidopsis database. The recombinant proteins were expressed in *E. coli*. By using an amino terminal his-tag the recombinant proteins were affinity purified and their pH-optima, substrate and regio specificities were determined. Moreover, their occurrence in different tissues from Arabidopsis was analyzed. Due to the involvement of LOX-derived products in signaling processes, we tested the transcriptional regulation of LOXs by sorbitol (leading to an endogenous rise of the plant homormone jasmonic acid), methyl jasmonate and salicylate.

4-56 CF-COI1 ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis

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F-box protein COI1 is required for response to jasmonates in Arabidopsis (Xie et al., 1998, Science 280, 1091-1094), which regulate root growth, pollen fertility, wound healing and defense against insects and pathogens. In this study, we demonstrated that COI1 physically associates with Arabidopsis Cullin AtCUL1, AtRbx1 and either of the Arabidopsis Skp1-like proteins ASK1 or ASK2 to assemble ubiquitin-ligase complexes, which we here designated SCF-COI1. COI1-E22A, a single amino acid substitution in the F-box motif of COI1, abolished formation of the SCF-COI1 complexes and resulted in loss of its function in jasmonate signaling. *AtRbx1* double-stranded RNA-mediated interference reduced the AtRbx1 level and dampened JA-inducible gene expression. Furthermore, we found that the AtCUL1 component of SCF-COI1 complexes is modified in planta. This modification is *AXR1*-dependent, and mutations in *AXR1* lead to a reduction in JA response. Finally, we demonstrated that the *axr1* and *coi1* mutations displayed a synergistic genetic interaction in jasmonate signaling. These results suggested that the *COI1*-mediated jasmonate response is dependent on the SCF-COI1 complexes in Arabidopsis, and that the *AXR1*-dependent modification of AtCUL1 subunit of SCFCOI1 complexes is important for JA signaling.

4-57 *KNAT2* displays complex interactions with cytokinin, gibberellin and ethylene

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The shoot apical meristem (SAM) produces the apical part of the plant. *KNOX* homeobox genes have been involved in the maintenance of the SAM, possibly via different signalling pathways. Recently, a tobacco KNOX protein has been shown to repress the expression of GA-20 oxidase, a key enzyme of gibberellin biosynthesis pathway. In this context, we are studying the link between *KNAT2* (*KNOTTED-like Arabidopsis thaliana*) and different hormones. The *KNAT2* gene is expressed in the SAM. As observed for other *KNOX* genes, overexpression of *KNAT2* led to a higher capacity to regenerate and delayed senescence, and the pattern of expression of *KNAT2* was enlarged in the presence of cytokinin. Thus, *KNAT2* is synergistic to cytokinin. Moreover, overexpression of *KNAT2* led to a reduction of GA-20 oxidase expression. Surprisingly, the phenotype of the *KNAT2* overexpressor was not restored in the presence of gibberellin, suggesting that the alteration of development was not due to a reduction of gibberellin content. We also investigated the relation with ethylene. The phenotype of the overexpressor of *KNAT2* was restored by ethylene. We showed that the overexpression of *KNAT2* antagonized the effects of ethylene in the SAM and the pattern of expression of *KNAT2* was negatively controlled by ethylene. Thus, *KNAT2* and ethylene are antagonists. The elucidation of the complete network between the signalling pathways and *KNOX* genes should give more insight in the comprehension of their role in the SAM.

4-58 Functional interactions of Arabidopsis abscisic acid signaling effectors in rice protoplasts

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We are elucidating the functions of genetically redundant gene families involved in abscisic acid (ABA) and stress signaling, especially the protein phosphatase 2C-like (PP2C), B3 domain, and bZIP domain regulatory factors whose cognates are the ABA-INSENSITIVE (ABI) genes *ABI1, ABI3,* and *ABI5,* respectively (1-4). We have previously shown in transient assays that ABI5 interacts synergistically with maize VIVIPAROUS-1 (*VP1*, the orthologue of *ABI3*) to transactivate ABA-inducible promoters from both moncots and dicots species, and that a dominant negative allele, *abi1-1*, can antagonize these effectors, demonstrating that ABA signaling pathways are conserved among higher plants (5). We are currently overexpressing cDNAs of 12 ABI1-like and 8 ABI5-like family members in transiently-transformed rice protoplasts and have thus far shown that the ABA-response element binding factors ABF1 and ABF3 (6) have novel activities in activating the ABA-inducible *Em* promoter of wheat. In the future we will characterize the antagonistic ABA activities of site-directed dominant-negative ABI1-like family members in rice and Arabidopsis, and characterize the physiology of available ABI-like knockout lines with a view to identify novel phenotypes amenable to further genetic dissection.

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4-59 The homeobox genes *ATHB7* and *ATHB12* are potential regulators of growth in response to drought in Arabidopsis

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

4-60 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. 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. We have determined that the WOL gene codes for a putative signal transducer with a histidine kinase activity (Mahonen et al. 2000: Genes&Dev. 14, 2938-2943). It is expressed specifically in the vascular tissue from the early stages of embryogenesis on. Recently, Inoue et al. (2001: Nature 409, 1060-1063) 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. In order to more systematically approach the genetic control of cell proliferation during vascular development, we have recently carried out an EMS based screen (~3200 M1 plants) for suppressors of wol based on root growth pattern. 13 strong and 7 weak suppressors were identified. Their genetic and anatomical analysis will be presented.

4-61 HDZip proteins are potential regulators of abscisic acid responsiveness in *Arabidopsis thaliana*

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The homeodomain leucine zipper (HDZip) transcription factors have been found in a range of different plants and they are unique to the plant kingdom. The Arabidopsis HDZip proteins, ATHB5, -6 and -16, are closely related and form a subgroup of HDZip I, one of the four major HDZip families. *In vitro*, ATHB5, -6 and -16 bind to pseudopalindromic DNA sequences; CAATNATTG, as protein dimers. Transient expression assays on Arabidopsis leaves demonstrate that ATHB5, -6, and -16 activate transcription of reporter gene constructs with upstream CAAT(A/T)ATTG binding sites, thus implying that ATHB5, -6 and -16 function as transcriptional activators *in vivo*. *ATHB5, -6* and *16* are all regulated by abscisic acid (ABA) at the transcriptional level, but in different manners. The phenotypes of plants overexpressing *ATHB5, -6* or *–16*, or carrying loss-of-function mutations in the genes, indicate that ATHB5, -6 and –16 act as regulators of different aspects of the growth response to ABA.

4-62 Cis-Jasmone as a novel signalling molecule in plant gene expression

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Jasmonic acid (JA) and its methyl ester (methyl-JA; MJA) are potent signalling molecules, belonging to a group of compounds collectively termed octadecanoids which are biosynthetically derived from the oxygenation of the plant fatty acid alpha-linolenic acid. JA and MJA are involved in diverse physiological processes such as promoting senescence, mechanotransduction, potato tuberization, with their role in defence reactions against herbivores and pathogens being extensively investigated. Interestingly, it has recently been demonstrated that JA and MJA are not the only active octadecanoids and that several other products from the lipoxygenase pathway have biological functions. (Z)-Jasmone (cis-jasmone) is a highly volatile product of JA catabolism and is not only a constitutive compound of flower volatiles but also induced by insect damage of vegetative tissue. It has been speculated that cis-jasmone might be a biologically-inactive sink for the JA pathway but recently we have demonstrated that cis-jasmone acts as an insect semiochemical (Birkett et al, PNAS 97: 9329-9334, 2000).
In an attempt to determine whether cis-jasmone is involved in defence signalling or whether it has additional functions we used the Stanford Arabidopsis DNA microarray for a large scale investigation of changes in gene expression by cis-jasmone treatment. We could show that cis-jasmone does act as a signalling molecule in Arabidopsis, that this signalling function is distinct from MJA and results obtained from preliminary microarray data analysis are presented here.

4-63 ABA-dependent activation of AREB bZIP transcription factors in drought- and high-salinity-stress signaling

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In order to study stress-signal transduction pathways during dehydration conditions, we have isolated and characterized genes that respond to water stress in Arabidopsis. The dehydration-responsive expression of the *rd29B* gene is mainly mediated by abscisic acid (ABA). We investigated two ABRE sequences in the *rd29B* promoter function as regulatory *cis*-elements, and isolated cDNAs encoding ABRE-binding bZIP proteins, AREB1, AREB2 and AREB3. Both AREB1 and AREB2 mRNAs accumulated in response to dehydration, NaCl and ABA treatment in roots and leaves, but weak expression of the AREB1 and AREB2 mRNAs observed in unstressed conditions. AREB1 and AREB2 were shown to transactivate the expression of a reporter gene fused to the ABRE motif in protoplasts, and require ABA addition for their maximum activation. In-gel kinase assay revealed that specific ABA-activated protein kinases phosphorylate the serine/threonine residues in conserved regions of the AREB proteins. Amino-acid substitution of the target serine/threonine residues to alanine resulted in the suppression of ABA dependent transactivation activity in protoplasts. These results suggest that the transactivation activity of the AREB proteins is regulated by ABA-responsive serine/threonine kinase. Properties of AREB phosphorylation will be discussed. On the other hand, the AREB genes themselves were also induced by drought, high-salinity, and/or ABA treatment, which suggests the induction of the AREB genes, is also important for their function in ABA-responsive gene expression. We then analyzed the promoter region of the AREB1 gene to identify *cis*-elements that function in drought and high-salinity responsive gene expression.

4-64 Gravity-regulated auxin transport from columella to lateral root cap cells

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Auxin gradients across the elongation zone (EZ) have long been discussed to mediate differential growth during root gravitropism (1). How these gradients are established and regulated in response to gravity, however, remains to be shown. We used *DR5-GFP* as an auxin biosensor to monitor auxin levels at cellular resolution during Arabidopsis root gravitropism. We identified elevated auxin levels in root cap columella cells, the site of gravity perception, and an asymmetric auxin flux from these cells to the lateral root cap (LRC) and the EZ in response to lateral gravistimulation. Cell to cell auxin transport involves uptake by diffusion or facilitated by influx carriers, and requires active efflux (2). We differentiated between an efflux dependent lateral auxin transport from columella to LRC cells, and an efflux and influx dependent basipetal transport from the LRC to the EZ. Furthermore, we demonstrated that gravity induced auxin gradients are established even under continuous supply of exogenous auxin. Live cell auxin imaging provides unprecedented insights into gravity regulated auxin flux at cellular resolution.

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4-65 *SPMS*, a paralog of *SPDS1/SPDS2* with spermine synthase activity

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Polyamines (PAs) have been implicated in the regulation of multiple processes during plant growth, including vegetative development, floral induction, and early fruit development. Based on physiological studies involving measurement of PA levels in various situations, the use of inhibitors of PA biosynthesis, and treatments with exogenous PAs, it has become apparent that the different PAs (mainly putrescine, spermidine and spermine) have differential roles during plant development. For instance, spermidine has been specifically associated with floral induction, while spermine levels have been linked to the decision between two fates for the ovary: senescence and fruit development. Therefore, we are interested in how PA biosynthesis is regulated and, in particular, how spermine levels are determined. In Arabidopsis there are two genes encoding spermidine synthase (*SPDS1* and *SPDS2*), and it has been reported that *ACL5* encodes a spermine synthase. We have isolated a gene, *SPMS*, which encodes another spermine synthase -as indicated by the complementation of yeast spermine synthase deficient mutant. However, the degree of identity between *SPMS* and *SPDS1/SDPS2*, as well as conservation of exon-intron structure suggest that *SPMS* is a paralog of *SPDS1* and *SDPS2*. Expression of *SPMS* was induced in seedlings by ABA treatments and, significantly, remained constant in unpollinated ovaries but decreased during early fruit development induced by gibberellic acid.

4-66 The Arabidopsis SERK2 receptor is involved in brassinolide perception

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The Arabidopsis *SERK1* gene encodes a receptor-like kinase (RLK) which enhances embryogenic competence in culture (Hecht et al., 2001). The SERK2 gene encodes a protein which is 90% identical in amino acid composition to SERK1 and contains exactly the same domains: an N-terminal signal sequence followed by a leucine-zipper domain, 5 LRRs, a proline rich "SPP" domain (the hallmark of SERK RLKs), a transmembrane domain, a functional kinase domain and a C-terminal leucine-rich domain. The *SERK2* cDNA was isolated from a cDNA library, and its expression pattern analysed by RT-PCR. Like *SERK1*, *SERK2* appears to be expressed during flower development. A *serk2* knockout mutant was isolated after remobilisation of a Ds element, and shows a dwarf "cabbage" phenotype typical of brassinosteroid (BR) mutants. The response of the *serk2* mutant to various BRs and other plant growth regulators was therefore tested. The mutant is insensitive to 22S-23S-homobrassinolide, and shows reduced sensitivity to 24-epibrassinolide. The phosphorylation properties of the SERK2 kinase domain were analysed by in vitro kinase assays. The SERK2-kinase domain shows intramolecular autophosphorylation but is unable to phosphorylate other proteins. The SERK2 receptor thus seems to be involved in BR perception and may function as a regulator of other proteins involved in BR signal transduction.

Hecht et al., 2001, Plant Physiology, 127, 803-816

4-67 Is AtWNK1 part of a signal transduction pathway regulating the V-ATPase?

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The V-ATPase is a ubiquitous eucaryotic proton pump, responsible for the acidification of endocompartments. As the pH of these compartments is a critical parameter that needs to be carefully controlled, we are trying to identify proteins involved in V-ATPase regulation. AtVHA-C (DET3), one of the V-ATPase-subunits was shown to interact with the novel protein kinase AtWNK1 in a Yeast 2 Hybrid (Y2H) screen.
AtWNK1 shows high sequence similarity to a recently identified family of mammalian kinases, which is characterized by the absence of a conserved Lysine residue and is therefore called WNK (**W**ith **N**o Lysine (**K**)) (Verissimo and Jordan, 2001).
To characterize the interaction between AtVHA-C and AtWNK1 on the molecular level and most importantly to test its role *in planta*, we are using the following approaches: A Y2H assay is under way to map essential sites of AtWNK1-VHA-C interaction by using randomly mutagenized AtWNK1. Kinase assays with purified AtWNK1 are used to confirm preliminary data suggesting, that *in vitro* translated AtWNK1 phosphorylates the V-ATPase subunit. Arabidopsis cell suspension cultures are used for expression and copurification of epitope-tagged AtWNK1 and AtVHA-C. V-ATPase activity will be analyzed in *AtWNK1* T-DNA mutants, as well as in wt and *det3* plants with altered AtWNK1 expression levels.
The identification of additional interaction partners for AtWNK1 together with the analysis of its expression pattern will hopefully allow us to connect AtWNK1 activity to known signal transduction pathways.

4-68 The use of *DR5-GFP* as an *in vivo* reporter for relative auxin contents in Arabidopsis plants

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In order to monitor relative auxin levels at cellular resolution, the *DR5-GFP* auxin reporter was generated. *DR5-GFP* consists of an ER (endoplasmatic reticulum) targeted mutated GFPm, driven by the synthetic auxin responsive promoter *DR5*. Transgenic Arabidopsis plants carrying single locus insertions of the *DR5-GFP* T-DNA were selected. Application of exogenous auxin to one-week old seedlings led to *DR5-GFP* induction in virtually all tissues of the Arabidpsis seedling after 1.5 hrs. In the absence of exogenous auxin, seedlings displayed strong GFP signals in the root tip, at the site of adventitious root initiation, and at the margins of cotyledons. In addition to GFP signals in root tips of main and adventitious roots, as well as cotyledon margins, adult plants exhibited localized strong fluorescence in tips of lateral roots, tips of primary and cauline leaves, dormant lateral buds, the seed funiculus, and guard cells of the upper leave side. Weaker GFP signals were often found in the vasculature of roots and hypocotyls. Confocal laser scanning microscopy of DR5-GFP seedling root tips allowed the identification of elevated auxin levels in defined cells of the quiescent center, columella initial and mature columella.

4-69 An AtPIN2-GFP fusion allows *in vivo* monitoring of AtPIN2 localisation during root gravitropism

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Basipetal auxin transport from the root tip to the elongation zone (EZ) is required for root gravitropism (1). The auxin efflux carrier protein AtPIN2 (2) (EIR1/AGR1) has previously been proposed to regulate this transport (1), and loss of AtPIN2 activity has been found to result in agravitropic root growth (2). We show that AtPIN2 is expressed in the lateral root cap (LRC), and is differentially regulated in response to gravity. We report on the generation and characterisation of a functional AtPIN2-GFP fusion, capable of complementing the *eir1-1/Atpin2* loss of function mutant. Laser scanning microscopy of transgenic Arabidopsis plants expressing AtPIN2-GFP, allows *in vivo* imaging of the protein at cellular resolution, and provides new insight in the regulation of root gravitropism.

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4-70 *PROPORZ1*, a likely component of a chromatin remodelling complex, is involved in the control of cell fate in response to auxin and cytokinin

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The growth regulators auxin and cytokinin exhibit an important impact on the control of cell fate in plant tissues. Concentration of and the ratio between these two hormones are critical parameters for the decision as to whether a cell will differentiate or whether it will re-enter the cell cycle. However, very little is known about the molecular network, which is integrating the stimuli of both hormones and is triggering the adequate responses.
We have isolated a mutant allele in a gene we termed *PROPORZ1* (*PRZ1*), which is altered in its response towards both growth regulators. When grown on exogenously applied auxin or cytokinin, *prz1-1* has a pronounced tendency to de-differentiate and to form callus-like tissue. Analysis of the mitotic cycB1-1::GUS construct revealed that cell proliferation in the mutant is conditional and strictly depends on increased auxin and cytokinin levels.
The yeast structural homolog of the PRZ1 gene represents a component of chromatin remodelling complexes, required for the expression control of target genes. We could show that PRZ1 exhibits transactivation activity in yeast, indicating a function for PRZ1 in gene expression control. Moreover, expression of PRZ1 appears to be most pronounced in meristematic tissue. Our current working model suggests that PRZ1 is involved in the establishment and/or maintenance of differentiation/de-differentiation processes via regulation of chromatin structure.
(This work is supported by grants from the FWF).

4-71 Functional analysis of GCR1 in *Arabidopsis thaliana*

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G-protein coupled signal transduction pathways are highly conserved in diverse eukaryotes. The first component of this signaling pathway is the G-protein coupled receptor (GPCR). GPCRs are 7 transmembrane receptors with an extracellular N terminus that perceives a signal and a cytoplasmic C terminus that couples with G-proteins to transduce the signal. In mammalian systems more than 1000 GPCRs can couple a variety of extracellular signals to their respective downstream pathways. In plants however, there is only one such prototypical receptor identified so far (1, 2). GCR1 from Arabidopsis has several conserved "domains" of GPCRs and shows significant homology to the cAMP receptor (CAR1) from *Dictyostelium*. Overexpression of GCR1 in tobacco BY2 cells and in Arabidopsis reduces flowering time and abolishes seed dormancy (3). Overexpressor plants bind more GTPγS and have higher rates of cell division, suggesting possible involvement in well-established G-protein signaling processes in plants (4). We have taken a genetic approach to functionally characterize GCR1 from Arabidopsis. We have screened for T-DNA insertion knockout mutants in this gene using the Arabidopsis knockout facility at University of Wisconsin. An initial characterization of the *GCR1* mutant phenotype and potential roles of GCR1 in stomatal function will be presented.

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4-72 Tracing the functional role of DOF transcription factors

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Dof-type proteins represent a recently identified novel type of transcription factors unique to higher plants. Dof factors have been shown to be involved in several biological phenomena, including seed development, hormone responses, and photosynthetic metabolism. Nuclear genome analysis has revealed that 38 Dof proteins are encoded by the Arabidopsis genome. We have started to investigate the functional role of 5 Dof proteins from this plant. cDNA clones were isolated and sequenced. The promoters of all five genes were cloned, fused to the GUS reporter gene, and transformed into plants to test for the cellular expression patterns. The results from these experiments will be reported. Overexpression of transcription factors often leads to phenotypic changes in transgenic plants that can not easily be explained as long as the target genes regulated by these factors are unknown. To facilitate the identification of target genes we are performing transcript expression profiling, using the Affimetrix microarray system, on wild-type and transgenic plants overexpressing selected Dof factors. Transgenic plants with suppressed level of mRNA (RNAi technique) were used to investigate the role of these genes in signal transduction pathways. To assist this type of analysis we are additionally performing metabolite profiling experiments (using gas chromatography / mass spectrometry) aimed at alterations in metabolite pathways. We will present and discuss data obtained so far using this type of approach.

4-73 The role of cytokinin signaling during secondary phase of vascular development

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Vascular tissue is first established during embryogenesis as an undifferentiated procambial tissue in the innermost domain of the plant embryo. Later in development, a lateral meristem (the cambium) is formed, as the undifferentiated cells begin to divide in the procambial tissue between the phloem and xylem strands. Therefore, the pattern of cell divisions is an important determinant of the cellular organization of vascular tissue in plants. We have recently shown that a recessive mutation, *wooden leg (wol)*, in the CRE1/WOL/AHK4 gene coding for a cytokinin receptor, results in reduced cell proliferation during procambial development in Arabidopsis root (Mähönen et al. 2000: Genes Dev 14:2938-2943). This indicates the involvement of a specific cytokinin mediated morphogenetic pathway during primary phase of vascular development.
Consequently, we have started to investigate the role of CRE1/WOL/AHK4 like receptors and cytokinin signaling during the secondary phase of vascular development. We have identified a putative cytokinin receptor gene family (Birch Histidine Kinase 2, 3 and 4) from a perennial tree species silver birch. Each of the three BHK genes are active in the cambial zone of the birch trunk. Furthermore, several other genes homologous to the members of various elements of the recently identified cytokinin core signaling pathway in Arabidopsis have been identified in an Expressed Sequence Tag collection derived from the cambial zone of birch trunk. Strategies for further functional analyses of the identified genes in Arabidopsis and in the tree system are presented.

4-74 Molecular cloning and characterisation of genes from Arabidopsis encoding cytochrome P450s of gibberellin biosynthesis

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Gibberellins (GAs) are diterpenoid carboxylic acids that function as hormones in higher plants. GAs were first isolated from the fungus Gibberella fujikuroi, from which gibberellic acid (GA3) is produced commercially. Although higher plants and the fungus produce structurally identical GAs, there are important differences in the pathways and enzymes used. In fact, higher plants and fungi have evolved the complex biosynthetic pathways to GAs separately and not by horizontal gene transfer.
Part of the GA-biosynthetic pathway (from ent-kaurene to GA53 in plants) is catalyzed by cytochrome P450s. We are studying these enzymes from *Arabidopsis thaliana* using G. fujikuroi and E. coli as heterologous expression systems. We are investigating the functional expression in E. coli of different Cyt P450s genes from Arabidopsis: ent-kaurene oxidase (KO) (CYP701A1), ent-kaurenoic acid oxidase (KAO) (CYP88A3 and CYP88A4), and a number of genes that are potential candidates for 13-hydroxylases. The bacterial signal peptide ompA was fused in-frame to these genes by PCR-mediated fusion. Translational fusions have been co-expressed with the Arabidopsis NADPH-Cyt P450 reductase (ATR1) and the enzyme activity was assayed using 14C-labeled substrates. We are also attempting to express AtCytP450 genes in mutants of G. fujikuroi lacking the GA-biosynthesis genes, which are present as a cluster of seven genes in this species.
Genes encoding the cyt. P450s that catalyze the 13-hydroxylation of GA12 to GA53 have not yet been isolated. Since developing cotyledons of Pisum sativum are a major source of 13-hydroxylase activity, we have isolated cyt. P450 cDNAs from this tissue by screening a subtractive cDNA library by PCR with degenerate primers, and are currently testing these and their Arabidopsis orthologues by heterologous expression.

4-75 Molecular characterization of the *AUXIN-RESISTANT6* gene

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The *auxin-resistant6* mutants show a variety of phenotypic alterations that implicate the *AXR6* gene in auxin response in essentially all tissues of the plant. *axr6* heterozygotes are short and bushy with a reduced number of lateral roots. However, we found no reproducible change in auxin transport in the *axr6* heterozygotes. *axr6* homozygotes arrest growth after germination, with either one or two cotyledons with vascular defects, sometimes a small number of true leaves, and no hypocotyls or root. We now present evidence that the *AUXIN-RESISTANT6* gene is identical to *AtCul1*, one of the genes encoding the protein cullin, a subunit of the ubiquitin ligase complex SCF. Mapping data place *AXR6* in an 80,000 bp region on the short arm of chromosome 4 containing 19 genes. One of these genes is *AtCul1*. Sequencing of *AtCul1* from two mutant *axr6* allele reveals that the identical base has been changed in both, producing two different amino acid changes in the cullin protein. Furthermore, crosses between *axr6* mutants and a knockout allele of *AtCul1* show that *axr6* does not complement the embryo lethality of the knockout. Additional tests of the identity between *AXR6* and *AtCul1* from transformation experiments will be reported. These results strongly support the developing model of auxin signal transduction, in which regulated protein degradation plays an essential role in auxin response.

4-76 Phosphate defective *pho3* mutant shows altered ABA- and sugar responses

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The uptake and transport of P is highly regulated and several groups are now investigating the mechanisms that regulate these fluxes of P using mutant approaches in *Arabidopsis thaliana*. The mutant pho3 was isolated on the basis of a failure to induce acid phosphatase activity in roots in response to low Pi. The pho3 mutant had a P deficient phenotype in vitro and in the leaves of soil grown pho3 plants the total P content was again reduced. pho3 exhibited a number of characteristics normally associated with low-P stress, including severely reduced growth, increased anthocyanin content and starch accumulation. Starch accumulation was initially thought to result from reduced leaf cytosolic Pi affecting flux through the phosphate translocator. However, the pho3 mutant was also found to accumulate extremely high levels of sucrose. This unexpected result suggested that pho3 was failing to respond normally to sucrose accumulation in photosynthetic tissue, and led us to investigate the sucrose responses of pho3 during germination. pho3 was shown to have an absolute requirement for exogenous sucrose as pho3 seedlings were arrested in early seedling development on sucrose-free medium and was unaffected by Pi-availability. pho3 was found to be insensitive to high concentrations of exogenous sucrose (10%) but was sensitive to glucose. pho3 also exhibited reduced sensitivity of seed germination to exogenous ABA (abi-phenotype), a response which has been linked to sucrose insensitivity in both sugar-sensing and ABA mutants. Hence ABA and sugar signals could contribute to the maintenance of phosphate homeostasis in normal plants.

4-77 OST1 kinase mediates the regulation of stomatal aperture by drought and abscisic acid

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During drought, the plant hormone abscisic acid (ABA) triggers stomatal closure, thus reducing water loss. Leaf temperature can be used as an indicator to detect mutants with altered stomatal responses, since stomatal aperture results in a rapid cooling of the leaf surface. We used infrared thermography to screen for Arabidopsis mutants that display a reduced ability to close their stomata in response to controlled drought stress. Some of the mutants recovered are deficient in ABA accumulation, whereas others are rather affected in ABA responsiveness. In particular, we isolated mutations at two novel loci designated as *OST1(OPEN STOMATA 1)* and *OST2* which reduce ABA responsiveness in stomata and not in seeds. Cloning and characterisation of the *OST1* gene will be described.

4-78 Point mutations in F-box protein COI1 lead to thermo-sensitive male fertility in Arabidopsis

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The *COI1* gene encodes a protein containing leucine-rich repeats and a degenerate F-box motif (Xie et al., 1998, Science 280: 1091). These structural features are characteristic of F-box proteins that function in ubiquitin ligase complexes for the ubiquitylation of substrate-proteins targeted for degradation. We recently demonstrated that COI1 assembles SCF- COI1 complex with Arabidopsis AtCUL1, AtRbx1 and SKP1-like proteins ASK1 and ASK2 to mediate jasmonate-regulated plant defense and fertility (Liu et al., 2002, 13th International conference on Arabidopsis Research). To further investigate structure-function relationship of COI1, we identified 15 *coi1* mutant alelles from a screen of about two million EMS-mutagenized M2 Arabidopsis seeds for jasmonate resistant mutants. Point mutations resulting from a single amino acid substitution in *COI1* were revealed in 11 mutants through DNA sequencing analysis. *coi1-2* and *coi1-8*, with missense mutations from Leu245 to Phe and from Glu543 to Lys, respectively, showed thermo-sensitive male sterility. When grown at 18-20ºC, *coi1-2* and *coi1-8* were fertile, more than 80% of flowers in the main bolts would develop sliques. However, when grown at 23-25ºC, *coi1-2* and *coi1-8* were male sterile and hardly produced viable pollen grains, only about 5% flowers in the main bolts could develop sliques. The yeast two hybrid screen with COI1 as bait has identified several interacting proteins including ASK1, ASK2, COIN3 and COIN4. The Glu543Lys mutation in *coi1-8* disrupted its association with COIN4, the remaining interacting proteins including ASK1, ASK2 and COIN3 were still able to interact with *coi1-8*. It would be interesting to test whether COIN4 interacts with COI1 in planta and whether COIN4 associates with *coi1-8* at low temperature (18-20ºC) in Arabidopsis.

4-79 The ethylene receptor ETR1 is part of a protein complex localized to the endoplasmic reticulum

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The ethylene receptor ETR1 of Arabidopsis is a modular protein with ethylene-binding, histidine kinase, and receiver domains. Sequence analysis does not provide information as to which membrane system of Arabidopsis the ETR1 ethylene receptor is localized. Examination by aqueous two-phase partitioning, sucrose density-gradient centrifugation, and immnuoelectron microscopy indicates that ETR1 is predominately localized to the endoplasmic reticulum (ER). Localization of ETR1 showed no change following a cycloheximide chase. Ethylene binding by ETR1 did not affect localization to the ER, based upon analysis of plants treated with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid and by examination of a mutant receptor that does not bind ethylene (etr1-1). Determinants within the amino-terminal half of ETR1 are sufficient for targeting to and retention at the ER. Gel filtration of solubilized ETR1 indicates that the receptor is part of a high-molecular-mass complex. Our data suggest that one role of the plant ER is to serve as a platform for receptor complexes in hormone perception and signaling.

4-80 Ppi1, a novel interaction partner for the plasma membrane H^+-ATPase

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Using the two-hybrid technique we identified a novel protein whose N-terminal 88 amino acids (aa) interact with the C-terminal regulatory domain of the plasma membrane (PM) H+-ATPase from *Arabidopsis thaliana* (aa 847-949 of isoform AHA1). The corresponding gene has been named *Ppi1* for *Proton pump interactor 1*. The encoded protein is 612 aa long and rich in charged and polar residues except for the extreme C-terminus, where it presents a hydrophobic stretch of 24 aa. Several genes in the *A. thaliana* genome and many ESTs from different plant species share significant similarity (50-70% at the aa level over stretches of 200-600 aa) to *Ppi1*. The PPI1 N-terminus, expressed in bacteria as a fusion protein with either GST or a His-tag, binds the PM H+-ATPase in overlay experiments. The same fusion proteins, as well as the entire coding region fused to GST, stimulate H+-ATPase activity. The effect of the His-tagged peptide is synergistic with that of fusicoccin (FC) and of tryptic removal of a C-terminal 10 kDa fragment. The His-tagged peptide binds also the trypsinized H+-ATPase. Altogether these results indicate that PPI1 N-terminus is able to modulate the PM H+-ATPase activity by binding to a site different from the 14-3-3 binding site and located upstream of the trypsin cleavage site. We are isolating knock out plants for both *Ppi1* and *2* genes in order to identify their physiological role.

4-81 Cross-talk between canopy-shade and auxin

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Previous studies implicated ATHB-2, a homeodomain-leucine zipper transcription factor, as a regulator of the shade avoidance response, which comprises a set of morphological changes that take place when the plant grows in a light environment with a low red:far red ratio (R:FR), and established a strong link between this factor and auxin signaling. On the basis of these findings, we postulated a model in which low R:FR light, via ATHB-2, induces a re-direction of auxin flux from the vasculature to external cell layers, resulting in a reduced auxin flux in the root.

Recent molecular data provided support to the model. Microarray experiments showed a significant increase in the level of several *IAA* mRNAs (*IAA2, IAA7, IAA19*) in wild-type plants after exposure to 4h FR-rich-light illumination (Arabidopsis Functional Genomic Consortium, Experiments 8130, 8266, 20902, http://genome-www4.standford.edu/Micro/Array/SMD). In addition, auxin levels were indirectly visualized in seedlings exposed to shade light using the synthetic DR5::GUS auxin reporter, whose activity correlates with direct auxin measurements. Consistent with the model, GUS expression was significantly increased in cotyledons and hypocotyl of DR5::GUS seedlings exposed to 4 h FR-rich-light illumination. Remarkably, cross-sections of hypocotyls revealed the GUS staining in the outer cell layers. The analysis of IAA gene expression in plants with elevated levels of ATHB-2 (35S::ATHB-2), exposed to shade light and in plants expressing an inducible-derivative of ATHB-2 (HD-Zip-2-V-G), treated with dexamethasone, is currently in progress. GUS expression analysis in 35S::ATHB-2 DR5::GUS plants is also in progress.

The first two authors contributed equally to this work.

4-82 GLABRA2 alters auxin responses during root epidermal cell patterning

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GLABRA2 (GL2) encodes a homedomain protein that belongs to the HD-ZIP IV family. Previous studies indicated that this gene is involved in trichome, root hair and seed-coat development. We took advantage of reverse and classic genetics to investigate the role of GL2 in root hair formation. We used a dexamethasone (DEX)-inducible system to generate transgenic plants that overexpress a fusion of GL2 with the hormone binding domain of the glucocorticoid receptor (G), under the control of the cauliflower mosaic virus 35S promoter. Following the activation of the GL2-G protein, the transgenic roots displayed a very severe reduction in root-hair initiation. Together with the inhibition of root hair formation, the transgenic roots showed alterations of several processes regulated by auxin, such as root elongation, gravitropic response and root hair elongation. Farmacological studies indicated that DEX-treated GL2-G plants behave as auxin- and ethylene-resistant mutants. A comparative analysis of newly-isolated *gl2* knock-out and leaky mutants, and plants entopically-expressing GL2 also revealed that this transcription factor regulates both root hair formation and elongation. Moreover, we showed that the lack of GL2 activity compensates the agravitropic phenotype of the *eir1* mutant. Taken together, the data strongly suggest that GL2 regulates root-hair differentiation processes through a modulation of auxin sensitivity in root epidermal cells. Latest experiments suggested that GL2 may alter auxin signaling in the epidermal cells through the regulation of cytoskeletal rearrangements and/or vesicle trafficking mediated by a specific PLD (see the abstract of Ohashi et al.).

4-83 Analysis of kinase function in *Arabidopsis thaliana* using gene knockouts

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Protein phosphorylation is involved in the regulation of many processes during the life cycle of a plant. In order to assign a function to specific kinase genes we employ a reverse genetics approach using T-DNA insertion mutants.
Our lab focuses on the function of calcium-dependent protein kinases (CDPK), CDPK-related protein kinases (CRK) and SnF-related protein kinases (SnRK). These gene families are comprised of 34, 8 and 39 isoforms, respectively. Arabidopsis T-DNA insertion mutants were obtained from three sources: Salk Institute Genomic Analysis Laboratory, Arabidopsis Knockout Facility at UW-Madison and Torrey Mesa Research Institute (San Diego, CA). So far, 49 gene knockout lines have been screened for homozygous plants. In the first phase of analysis homozygous lines are then screened for phenotypical changes during growth and development under optimal conditions. In the second phase all lines are subjected to different environmental stresses. Tested conditions include cold, heat, high salt, high sugar and pathogen infection.
Details of screening procedures and recent results will be presented.

4-84 Hormone interactions in elongation control of hypocotyl and root of light-grown Arabidopsis seedlings

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We investigated the interaction of auxin, ethylene and cytokinin on the level of individual elongating cells in the epidermis of hypocotyls and roots. In darkness auxin, ethylene and cytokinin all decrease hypocotyl elongation. In the light the published data on hypocotyl length are contradictory. We found differential and specific effects for each hormone. In the light auxin promoted cell elongation at the base of the hypocotyl but inhibited elongation at the top. The total hypocotyl length was ultimately not affected. Ethylene strongly promoted cell elongation in the top part of the hypocotyl, leading to a greater overall length of the organ. When seedlings were grown on low-nutrient medium instead of normal Murashige and Skoog medium, the hypocotyl length was reduced due to an elongation block in cells in the middle part of the hypocotyl. Exogenous auxin or ethylene could both restore this defect in elongation, which could point to a possible co-action between auxin and ethylene under this growth condition. Also cytokinin could rescue the elongation when it was reduced by blocking auxin transport or ethylene action. Our results indicate a very complex interrelation of hormones during elongation control in hypocotyls.
In roots the situation seems to be simple: both auxin and ethylene have a fast and negative effect on elongation. Experiments using several lines of transgenic plants with auxin-responsive domain and b-glucuronidase (GUS) fusions, suggest however that both hormones act separately and in parallel on cell elongation.

4-85 Protein degradation in JA-signaling

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The focus of our research is to understand the role of oxylipins such as jasmonate (JA) in the plant defence response. To assess the role of JA in both local and systemic wound-induced gene activation, a search for novel wound- and JA-inducible genes has been undertaken in *Arabidopsis thaliana* (Titarenko et al. 1997. Plant Physiol. 115, 817-826). Some genes (JR1, JR2 and JR3) were shown to be strongly induced by wounding and JA, while others (WR3 and ACO) were induced by wounding only, suggesting that separate JA-dependent and Ðindependent wound signal transduction pathways exist in Arabidopsis (Leon et al. 1998. MGG 258, 412-419). *COI1*, an F-box protein, has been shown to be a key componant of the JA-dependent signal transduction pathway. This protein shares similarity to the F-box proteins Arabidopsis *TIR1*, human *SKP2*, and yeast *GRR1*, which appear to function by targeting repressor proteins for removal by ubiquitination (Xie et al. SCIENCE 280, 1091-1094). According to those similarities, we have undertaken the characterization of the expression of wound- and JA-inducible genes, following a treatment with MG132, a proteasome inhibitor. Our preliminary results tend to demonstrate the involvement of the proteasome in JA-dependent signaling. It is tempting to assum that *COI1* could direct the specific ubiquitination of a JA-dependent pathway repressor, further leading to a derepression of this pathway. A proteomic approach to uncover the targets for ubiquitination is currently underway.

4-86 Gibberellin signal transduction and three *RGA*-like genes

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The previously characterized *RGA* and *GAI* genes encode negative regulators of gibberellin (GA) signal transduction. RGA and GAI are members of the GRAS family of putative transcriptional regulators. Unlike other GRAS family members, however, RGA and GAI contain a DELLA motif, which appears to be necessary for the GA-responsiveness of these proteins. Database searches have recently identified three other Arabidopsis genes that encode proteins with sequence similarity to GAI and RGA. The three new DELLA sub-family members have been named *RGA-like 1, 2,* and *3* or *RGL1, RGL2*, and *RGL3*. We have identified T-DNA insertions in the *RGL* genes. Currently, we are studying the effects of these mutations, both singly and in combination with each other. Our genetic analysis is an attempt to determine the specific role of each *RGL* gene in GA signaling, while also examining the possibility of redundancy of function between the *RGL* genes, *RGA*, and *GAI*.

4-87 Analysis of *ETHYLENE INSENSITIVE6* and the *ENHANCER OF ETHYLENE INSENSITIVITY*

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

4-88 Microarray-based analysis of auxin response in Arabidopsis roots identifies novel early auxin response genes

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We used custom-made cDNA microarrays to compile a list of genes that are upregulated by auxin in Arabidopsis roots. Plants were grown on nylon rafts on sterile media for 14 days and then transferred to sterile water or water plus 10 µM IAA for a period of 45 min to 12 h. RNA isolated from these roots was used as a template to probe our 12,000 element microarrays. After normalization, we identified a large number of genes that are reproducibly upregulated by auxin. As expected, this list contains many known auxin response genes including, among others, 5 members of the *IAA* gene family, *SAUR*, *GH3*, and *FQR1*. These serve as positive controls. Also as expected, the number of genes showing upregulation by IAA increases with the length of auxin treatment. Some of the auxin upregulated genes code for proteins of unknown function. Others code for proteins of known classes, such as homeodomain proteins, whose pathways are not yet directly linked to auxin response. A third group includes genes whose protein products may participate in previously identified pathways for auxin response, including the ubiquitin-mediated protein degradation pathway. One of the novel auxin regulated genes whose differential expression has already been confirmed by northern analysis contains an F-box motif similar to that found in TIR1.

4-89 *ETHYLENE RESPONSE DNA-BINDING FACTOR1*: A novel downstream regulator of the ethylene response in Arabidopsis

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The response of plants to phytohormone ethylene involves a very complex combination of developmental and physiological processes and is mediated through the changes in the expression levels of hundreds of genes. Two main ethylene response DNA elements have been previously characterized in Arabidopsis. The EIN3-binding site, the primary ethylene-response element, is recognized by the members of the EIN3 family of transcription factors. Binding to the secondary ethylene-response element, the GCC-box, is accomplished by the AP2-domain-containing proteins collectively known as EREBPs. However, a significant proportion of ethylene-regulated genes lacks either of these two elements implying that other, yet unknown, transcription factors may be responsible for the activation/repression of these genes. In order to identify these novel components and to determine which branches of the ethylene pathway they regulate, we focused on the signaling events that occur following the EIN3/EIL protein activation. Herein, we report identification and characterization of *EDF1*, an immediate-early component of the downstream branch of ethylene response that mediates morphological effects of the ethylene gas. EIN3 binds to the promoter of *EDF1* and activates its transcription. The EDF1 protein, in turn, binds to the defined sequence in the promoters of its target genes, triggering a subset of ethylene responses. Mutant analysis indicates that *EDF1*, along with its three paralogs, is required for the normal sensitivity of plants to ethylene. The results of overexpression studies are consistent with the downstream position of the *EDF* family members in the ethylene signaling pathway.

4-90 Arabidopsis brassinosteroid-insensitive dwarf12 mutants are semi-dominant and have mutations in a GSK3β like kinase

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The plant steroid hormones Brassinosteroids (BR) regulate many aspects of plant growth and development. The plasma membrane-located BR receptor BRI1 has been shown to be a mediator of BR signals; however, a downstream cascade of BR signaling has yet to be discovered. We have isolated two novel BR-insensitive mutants *dwarf12-1D* and *dwf12-2D*. *dwf12* mutants display the characteristic morphology of previously reported BR dwarfs, such as short stature, sterility, and abnormal de-etiolation as well as a unique phenotype including severe downward curling of the leaves. Genetic analysis indicated that the two mutations are semi-dominant in that heterozygous plants show an intermediate phenotype whose height is between wild-type and homozygous mutant plants. Unlike BR biosynthetic mutants, *dwf12* plants were not rescued by high doses of exogenously applied BRs. Like *bri1* mutants, *dwf12* plants accumulate a significant amount of BRs including castasterone and brassinolide, providing further evidence that *dwf12* is a component of the BR signaling pathway. Map-based cloning of *DWF12* revealed that DWF12 belongs to the shaggy/GSK3 (glycogen synthase kinase 3) kinase family. Interestingly, yeast two hybrid analyses showed that DWF12 does not directly interact either with BRI1-kinase domain or recently identified TWISTED whose mutant protein confers BR insensitivity. Based on the *DWF12* overexpression phenotypes, sequence analysis, and yeast experiments, we propose that *dwf12* mutants are gain-of-function mutations and DWF12 acts downstream of BRI1 receptor as a naturally active repressor of BR signaling pathways.

4-91 Brassinosteroid signaling in Arabidopsis

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Brassinosteroids (BRs) are essential hormones for normal plant growth and development. BR signal transduction is mediated by the cell-surface receptor kinase BRI1, which initiates a signal transduction cascade leading to nuclear gene expression and cell elongation. BR responses are negatively regulated by the GSK3/SHAGGY-like kinase BIN2. A genetic screen for mutants insensitive to the BR biosynthetic inhibitor brassinazole has led to the identification of the dominant mutant brassinazole resistant1 (bzr1-1D). The bzr1-1D mutant has increased cell elongation in the dark but reduce cell elongation in light due to increases feedback inhibition of BR biosynthesis. The bzr1-1D mutation suppresses det2, bri1 and bin2 mutants. BZR1 encodes a nuclear protein whose abundance is increased by BR treatment and the bzr1-1D mutation. BR treatment induces dephosphorylation prior to accumulation of the BZR1 protein. BIN2 directly interacts with BZR1 in yeast two-hybrid assays, phosphorylates BZR1 in vitro, and negatively regulates BZR1 protein accumulation in vivo. Experiments using a proteasome inhibitor suggest that phosphorylated BZR1 is degraded by the proteasome machinery. These results strongly indicate that BIN2 phosphorylates BZR1 and targets BZR1 for degradation by the proteasome, and that BR signaling mediated by the receptor kinase BRI1 causes BZR1 dephosphorylation and accumulation, most likely by inhibiting BIN2 activity. T-DNA insertion lines of bzr1 and its homolog bzr2/bes1 have been identified and double mutants are being generated and analyzed. Additional BZR1-interacting proteins have been identified. A model of BR signal transduction pathway will be discussed.

4-92 Genetic analysis of the *ade1* ABA response mutant

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Because hormone-signaling pathways in plants are likely to be very complex classical mutant screens such as germination assays have only been able to identify a few ABA signaling intermediates. To circumvent this limitation we have employed a firefly luciferase reporter gene mutant screen to identify EMS-generated mutants that exhibited altered ABA-responsive gene expression patterns. After three rounds of luciferase screening and RNA gel blot and genetic analyses we identified at least 30 candidate mutant loci. *ade1*, a monogenic recessive mutant, exhibits a desensitization response and an amplitude response although in most visible aspects the plants appeared normal. Physiological and genetic studies were undertaken to confirm whether *ade1i* was a bona fide ABA response mutant. Osmotic potential determination, freezing tolerance assays, and leaf transpiration assays indicate that the *ade1* mutant has enhanced water relations as compared to the wild type. Double mutant analyses with known ABA response mutants indicates that the *ade1* mutation is epistatic to both the *abi1* and *aba3* mutations. These data will be discussed with reference to the possible function of the mutant *ade1* locus as identified by positional cloning.

4-93 Loss of nonhost resistance of Arabidopsis *nahG* to *Pseudomonas syringae* pv *phaseolicola* is not due to absence of salicylic acid and may be an effect of catechol

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Salicylic acid (SA) acts as an endogenous signaling molecule that is involved in plant defense against certain pathogens. Transgenic *nahG* plants harbor the bacterial *nahG* gene encoding salicylate hydroxylase, which converts SA to catechol. *nahG* plants accumulate only very small amounts of SA and have been very helpful in providing evidence for the requirement of SA for resistance to certain pathogens. Recently, Arabidopsis *nahG* plants were shown to have lost their nonhost resistance to *Pseudomonas syringae* pv *phaseolicola* NPS3121 (*Psp*), suggesting that nonhost resistance is controlled by the SA-mediated defense pathway. However, we tested *Psp* on various known SA signaling mutants and found that bacterial multiplication was arrested in SA mutants as it was in wild-type plants, indicating that the nonhost resistance was not dependent on the SA signaling pathway. Consistent with the previous report, we found that the bacteria multiplied in *nahG* plants. A double mutant containing the SA-biosynthetic mutation *sid2* and the SA-converting transgene *nahG* was found to retain its nonhost resistance to *Psp*, indicating that the susceptibility of *nahG* plants to *Psp* results from its SA-degrading activity. Catechol, the breakdown product of SA by NahG, was found to induce susceptibility to *Psp*, which suggests that the lack of nonhost resistance of *nahG* plants to *Psp* may be due to catechol accumulation. Based on this data, we advise using SA-deficient mutants other than *nahG* plants to study the role of SA in biological phenomena.

4-94 Molecular characterisation of the *GA20 oxidase-1* promoter from Arabidopsis: Identification of *cis*-elements that mediate control of gene expression by gibberellins

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Gibberellins (GAs) are a class of plant hormones that are involved in a wide range of developmental responses such as germination, elongation growth and flowering. The processes regulated by GAs are, at least in part, limited by the concentration of biologically active GAs, and therefore the regulation of GA biosynthesis is an important factor in the control of plant development. GAs are the products of a long, tightly regulated, biosynthetic pathway, whose later part is catalysed by 2-oxoglutarate-dependent dioxygenases including the enzyme GA 20-oxidase. *GA20ox* genes are down-regulated by bioactive GAs in a feedback mechanism that maintains the concentration of GAs within plant tissue. The regulation of genes encoding GA 20-oxidases is, therefore, of fundamental importance in the control of GA levels and plant development. A major part of the feedback control over GA 20-oxidase gene expression operates at the level of transcription, as shown by the analysis of Arabidopsis transgenic plants containing a construct in which the *AtGA20ox1* promoter was translationally fused to the *gus* cassette. The reporter gene is expressed at low levels in seedlings but treatment with paclobutrazol, an inhibitor of GA biosynthesis that acts early in the pathway and reduces bioactive GA levels, results in marked up-regulation, faithfully reflecting the expression pattern of the endogenous *AtGA20ox1* gene and its feedback-regulation by GA. Since feedback control of the *AtGA20ox1* gene operates at the transcriptional level, we have started to dissect the molecular mechanisms that regulate transcription. Analysis of a 5-end deletion series of *AtGA20ox1* promoter in transgenic plants demonstrates that the sequence elements within a 379 bp proximal promoter fragment are enough to confer tissue-specific expression and response to GAs. In vivo genomic footprinting of the *AtGA20ox1* promoter reveals differences in the DMS-methylation patterns between two week old seedlings and mature pollen. Differentially methylated **G**-residues overlap near-palindrome and direct repeat motifs previously identified by sequence analysis. These sequence motifs are, therefore, prime candidates for regulatory elements that control *AtGA20ox1* gene expression and possibly mediate the feedback response to GAs.

4-95 Cloning and characterization of the phosphatidylinositol-specific phospholipase C (PI-PLC) from *Brassica napus*. The effect of its over-expression on transgenic canola lines

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It is becoming increasingly evident that, like mammalian and other systems, plants sense, and appropriately respond to a variety of biotic and abiotic stress factors through comparable signal transduction mechanisms. In many cases the signal transduction cascades appear to require the activation of specific phospholipases e.g. PLA, PLD and PLC. For example, the production of the well documented mammalian second messenger, inositol 1,4,5-trisphosphate (IP3) by phosphatidylinositol bisphosphate (PIP2)-specific PLC has been shown to be involved in the process of osmoregulation in plants. Furthermore, PI-PLC mediated cascades in higher plants maybe cell type-specific, as in the case of guard cells, where PI-PLC and PLA were shown to work antagonistically to regulate stomatal movement through the transient modulation of IP3 levels.
In this presentation we report on the cloning and characterization of the PI-PLC gene isolated from Brassica napus, cv Westar. Its tissue-specific distribution and the effect of different abiotic stress conditions on its transcription levels in canola will be discussed. We also report, for the first time, on the effect of over-expression of this enzyme in transgenic *B. napus*.

4-96 Gibberellins insensitive phenotype conferred by over-expression in *Arabidopsis thaliana* of a strawberry GAST like gene

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A gene (*FaGAST*) was identified in strawberry. The deduced 106 amino acid sequence of FaGAST shares high similarity with previously characterised putative cell wall proteins of unknown function. These proteins are encoded by gibberellin inducible genes, namely *GASA* (for GA-stimulated) in Arabidopsis, *GAST1* (for GA stimulated transcript) from tomato, *GIP* (for GA induced gene) in petunia and the auxin inducible gene *RSI-1* (for Root System Inducible-1) from tomato. The corresponding peptides display similar structural features: a putative signal peptide in the N-terminal domain, a highly divergent central region and a conservative 60 amino acid C-terminal domain containing 12 cysteine residues which defines a pattern not related to other known cysteine-rich motifs.
Over-expression of *FaGAST* in *Arabidopsis thaliana* produced dwarfism, certain insensitive to high concentrations of GA3 and a delay in flowering time.
Our studies suggest that *FaGAST* plays an important role in GA-linked cell wall development.

4-97 bZIP factors involved in stress-responsive ABA signaling

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Phytohormone abscisic acid (ABA) plays an essential role in adaptive responses of plant to various environmental stresses such as drought, high salt and cold/freezing. The hormone regulates, among others, the expression of numerous stress responsive genes. From various promoter analyses, cis-regulatory elements known as ABREs (Abscisic acid Responsive Elements) have been determined, and a number of their putative cognate trans-acting factors have been isolated based on their interaction with the elements. To date, however, in vivo data showing their involvement in ABA/stress signaling are still lacking. Here, we report that ABRE binding factors, ABF3 and ABF4, function in ABA signaling in planta. Constitutive overexpression of ABF3 or ABF4 in Arabidopsis resulted in ABA hypersensitivity and other ABA-associated phenotypes that include hypersensitivities to salt and sugar. Importantly, the transgenic plants exhibited reduced transpiration and enhanced drought tolerance. At the molecular level, altered expression of a number of ABA/stress-regulated genes was observed. Furthermore, the temporal and the spatial expression patterns of ABF3 and ABF4 were consistent with their suggested roles in stress response. Together, our results provide strong in vivo evidence that ABF3 and ABF4 mediate stress responsive ABA signaling.

4-98 Novel brassinosteroid receptors function in vascular development

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Plant steroid hormones are perceived at the cell surface by BRI1, a LRR-receptor protein kinase. Binding of the steroid to the receptor activates a signal transduction mechanism that controls a variety of developmental processes, such as cell elongation and differentiation. Three Arabidopsis *BRI1* homologues were identified, and named *BRL1* (*BRI1 Like*), *BRL2* and *BRL3*. We decided to investigate whether these proteins function as brassinosteroid receptors and their role in plant development.
Promoter-GUS fusions of *BRL1* and *BRL3* showed a complementary pattern of expression that is specific for vascular tissues. Using different T-DNA insertion collections, we identified knock-out mutants for each of these genes in two different ecotypes and performed a phenotypic characterization of them. The *brl1* mutant was found to exhibit altered organization of vascular tissue in the inflorescence stem. In parallel, we examined BR-deficient (*det2*) and insensitive (*bri1*) mutants, and found that the phloem:xylem ratio is altered. These defects are enhanced in the different double and triple mutant combinations.
Expression of *BRL1* and *BRL3* under the control of the *BRI1* promoter recapitulated *bri1* mutant phenotype. Finally, we have performed 3H-brassinolide binding assays, which show that these proteins function as brassinoteroid receptors. Based on these findings, the role of these novel brassinosteroid receptors in regulating vascular cell differentiation in Arabidopsis will be discussed.
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4-99 Potassium transporter TRH1 functions as a regulator of auxin transport in Arabidopsis roots

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Tiny Root Hair 1 from *A. thaliana* encodes a protein belonging to the multi gene family of KT/KUP/HAK potassium transporters. *trh1* mutant has been scored from a population of T-DNA insertion lines by an aberration in root hair development. Further analysis of *trh1* phenotype revealed that this mutation is also associated with a reduced gravitropic response, suggesting an involvement of TRH1 protein in auxin-dependent signalling and/or auxin transport. A role of TRH1 in auxin transport was assessed with use of radio-labelled auxin 3H-IAA. After incubation in a solution containing 3H-IAA, *trh1* root segments accumulated more radioactivity as compared to wt plants. When root segments loaded with 3H-IAA were washed in auxin-free solution a rate of radioactivity efflux was higher in wild type plants. This experiment suggest that TRH1 facilitating auxin efflux can control auxin distribution in root tissue. To test this hypothesis we studied expression of GUS gene cloned under synthetic auxin responsive elements DR5. Analysis of GUS expression in DR5::GUS transgenic plants revealed that auxin distribution indeed is affected by *trh1* mutation wich lead to strong accumulation of auxin activity in the central cylinder. Finally, exogenously applied auxin rescued gravitropic response and root hair growth in trh1 mutant. All these lines of evidence strongly indicate that TRH1 protein functions as an regulator of auxin transport in Arabidopsis roots.

5-01 Reproductive development in *Arabidopsis*

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We recently identified a putative chromatin remodeling factor, *SPLAYED* (*SYD*) as a genetic enhancer of a weak *leafy* mutant, *lfy-5* (1). *SYD* encodes one of four SWI/SNF ATPases of the Snf2p subgroup in *Arabidopsis*. This subgroup of ATPases was found to regulate transcription in many different organisms from yeast to man by altering the nucleosome-DNA interactions in promoter regions. While the molecular mechanism of action of Snf2p ATPases has recently been the focus of intense investigation, the role these proteins play in multicellular eukaryote development is unclear because mutations in the SNF2p-like ATPases are generally embryo-lethal. The null allele of *SYD* is viable and thus allows us to characterize the role of this Snf2 ATPase in *Arabidopsis* development. Previous (1) and current investigations indicate that *SYD* regulates several distinct developmental pathways in apical as well as axillary meristems: The *syd* single mutant exhibits defects in shoot apical meristem (SAM) maintenance during the reproductive phase, resulting in SAM termination. Genetic investigation revealed an earlier role of *SYD* in the SAM duringembryo development. *SYD* also regulates aspects of SAM identity. In addition, SYD is required in the *lfy* mutant but not in the wild-type for class B and class C floral homeotic gene expression, suggesting that *SYD* acts as a redundant co-regulator of floral homeotic genes in the floral meristem.

1. D. Wagner, E. M. Meyerowitz, Curr Biol 12, 85-94. (2002)

5-02 Regulation of floral homeotic gene expression by a putative transcription co-repressor complex

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Proper regulation of floral homeotic gene expression is critical for floral pattern formation. To understand how the floral homeotic gene *AGAMOUS* (AG) mRNA is only expressed in the center of an Arabidopsis floral meristem to specify stamen and carpel identity and to repress further proliferation of the floral meristem, we have identified and characterized three negative regulators of *AG*: *LEUNIG (LUG), SEUSS (SEU)*, and *LARSON (LSN)*. Mutations in all three genes cause ectopic and precocious expression of *AG* mRNA, leading to partial homeotic transformation of floral organs in the outer two whorls as well as a reduction of floral organs. *lug; seu* and *lug; lsn* double mutants exhibit an enhanced phenotype with a more complete homeotic transformation of floral organs and a greater extent of floral organ loss. In situ hybridization and double and triple mutant analyses showed that this enhanced defect was caused by an enhanced ectopic and precocious *AG* expression. All three genes have been isolated using a map-based approach. *LUG* encodes a putative transcriptional co-repressor with sequence similarity to the yeast co-repressor Tup1. *SEU* encodes a novel protein with at least two glutamine-rich domains and a highly conserved dimerization domain. *LSN* encodes a homeodomain protein with sequence similarity to the *Arabidopsis* ovule developmental regulator *BELL1*. *LUG* physically interacts with *SEU* and *LSN* in the yeast two-hybrid assay. In addition, *LSN* binds directly to the *AG* cis-regulatory element as shown by the electrophoretic mobility shift assay. We propose that *LUG* and *SEU* may form a co-repressor complex, which is recruited to the *AG* cis-elements by *LSN*.

5-03 *BRACTS* promotes bract formation on *Arabidopsis* flowers

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In *Arabidopsis thaliana* and most members of the Brassicaceae family, transformation of the vegetative clade into the floral clade involves the activation and repatterning of the side shoot into a flower. At the same time, the subtending leaf (bract) is completely inhibited. Understanding the mechanism by which bracts are inhibited in Brassicaceae may provide important insight into the evolution of inflorescence architecture as well as the regulation of leaf development, but little is known about the underlying mechanisms. To understand bract inhibition we are characterizing an activation-tagged line termed *bracts-1d* (*brx*), in which development of bracts is not inhibited. In addition to defects in bract inhibition, *brx* mutants also develop petiole-less leaves and, in homozygous plants, develop leafy shoots in place of flowers. Expression analysis shows *BRX* to be expressed in morphogenetic tissues including meristems, young leaves, and floral organs. Double mutant combination with *apetala1* (*ap1*) dramatically enhances the *brx* phenotype, suggesting that *BRX* and *AP1* act on the same pathway. Using the *AP1* promoter to lower expression via RNAi in the flower results in shorter sepals and petals, while using this same promoter to drive expression of *BRX* results in the fusion of sepals to each other and, in strong lines, to the complete inhibition of floral organ development. These results suggest that *BRX* may be involved in maintaining an undifferentiated state required for morphogenesis. Thus, misregulation of *BRX* may lead to ectopic morphogenesis or the inhibition of differentiation itself.

5-04 The *PETAL LOSS* gene of Arabidopsis

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In *petal loss* (*ptl*) mutant plants the number of petals is reduced and they are often mis-shapen and mis-oriented. Also, sepals are boat-shaped and sometimes fused to each other. In order to understand better the genetic mechanisms of *Arabidopsis* perianth development, we isolated the *PTL* gene and analysed its normal expression patterns and the consequences of its ectopic expression.
A map-based approach was used to clone *PTL*. The wild type copy of this gene was able to fully restore the *ptl* mutant phenotype, and five *ptl* alleles each contain a significant defect in the PTL coding sequence. *PTL* is a member of the GT transcription factor family so far found only in plants. GT factors have either one or two trihelix DNA binding domains, distantly related to Myb DNA binding domains. Trihelix domains were discovered in proteins that bind to GT elements found in the promoters of many light responsive genes. *PTL* has two trihelix binding domains and is the first member of this family associated with a mutant phenotype.
Northern and in situ hybridisation tests indicated that *PTL* RNA transcripts are very rare, but RT PCR experiments detected significant expression in inflorescences. The *PTL* promoter drives GUS reporter gene expression within the flower primordium in four defined regions between the sepals prior to petal initiation. GUS expression was also detected in the margins of developing sepals, and also in several other floral and vegetative regions unaffected in *ptl* mutant plants.
Plants transformed with p35S::PTL were severely stunted if they grew at all. Consistent with this, expression of *PTL* using floral specific promoters in the LhG4/pOP trans-activation system showed arrested growth in tissues where *PTL* was ectopically expressed. This indicates that *PTL* may function as an inhibitor of growth. In this way, *PTL* may normally act in young flower buds to limit the growth between sepal primordia, allowing them to arise separately, and providing a limited domain for petals to arise. In *ptl* mutants, signals that control the initiation of petals may be weakened, resulting in the initiation of fewer petals than normal. Also, a signal controlling their orientation may be disrupted. Fundamental questions remain about what ultimately causes organs to initiate growth, and how an organ perceives its position within the flower.

5-05 *STY1* and related genes promote the formation of apical tissues during Arabidopsis gynoecium development

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Gynoecium ontogenesis in Arabidopsis is accomplished by the coordinated activity of genes that control patterning and the regional differentiation of individual tissues, and ultimately results in the formation of a basal ovary, a short style and an apical stigma.
stylish1-1 (sty1-1) mutants are essentially wild-type-like but develop reduced amounts of stylar and stigmatic tissues, concomitant with reductions in stylar xylem, resulting in an aberrant style morphology. Constitutive expression of *STY1* in transgenic Arabidopsis causes ectopic formation of style cells in place of valve cells. This suggests a role for *STY1* in promoting the formation stylar and stigmatic tissues. Consistently, *STY1* is expressed in the apical parts of the developing gynoecium.
STY1 belongs to a family of 10 genes, the members of which encode proteins with a cysteine-histidine rich domain similar to the zinc binding RING finger domain. Double and triple mutants between *sty1-1* and plants with mutations in *STY* related genes, showing no apparent gynoecial defects, display enhanced *sty1-1* phenotypes with more severe reductions in stylar and stigmatic tissues as well as in stylar xylem, suggesting redundant functions among the members of the gene family. Double mutants between *sty1-1* and plants mutant for other style promoting genes indicate that *STY1* genetically interacts with *SPATULA* and possibly also with *CRABS CLAW*.

5-06 The Arabidopsis hybrid kinase CKI1 is essential for megagametogenesis

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CKI1 (cytokinin-independent) was the first non-ethylene receptor histidine kinase discovered in Arabidopsis. The putative protein structure, combined with the observation that Arabidopsis callus tissues overexpressing CKI1 exhibit a "cytokinin-independent" cell division and greening phenotype (1), led to the hypothesis that CKI1 is involved in cytokinin signaling, perhaps acting as a cytokinin receptor. The putative extracellular ligand-binding domain of CKI1, however, is quite different from that of the identified cytokinin receptor family (CRE1, AHK2, AHK3), sharing only about 5% identity at the amino acid level. Furthermore, expression of CKI1 in Arabidopsis protoplasts (2), E. coli lacking the histidine kinase RcsC (3), or budding yeast lacking the histidine kinase Sln1 (pers. comm.), has been shown in all cases to activate cytokinin-inducible histidine kinase signaling pathways in a constitutive, rather than cytokinin-dependent, manner. Thus, questions remain regarding the role that CKI1 may play in hormone signal transduction. To rigorously test the function of CKI1 in planta, we have used a reverse genetic approach to identify plants containing a null allele of CKI1. Based on segregation distortion, transmission studies, mRNA expression data, and a microscopy-based examination of developing female gametophytes, we suggest that CKI1 function is required for megagametophyte development in Arabidopsis. These results as well as current progress toward elucidating the in situ functions of this hybrid histidine kinase will be presented.

1. Kakimoto, T. (1996) Science, 274: 982-985
2. Hwang, I. & Sheen, J. (2001) Nature, 413: 383-389
3. Yamada et al. (2001) Plant Cell Physiol, 42: 1017-1023

5-07 Dissection of multiple roles of UFO during floral development by ethanol inducible *UFO* expression

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The *UNUSUAL FLORAL ORGANS (UFO)* gene is required for several aspects of floral development in Arabidopsis including specification of the identity of the second and third whorls organs, the petals and stamens, and the proper pattern of primordium initiation inside the first whorl. *UFO* is expressed in a dynamical pattern during the early phases of flower development. Here we dissect the timing of UFO role during flower development by expressing UFO in ufo-2 mutant flowers at different developmental stages and for various duration by using an ethanol-inducible expression system. Beside fully complemented flowers, novel floral phenotypes were observed which were not seen in loss-of functions or constitutive gain-of-function of *UFO*. We show that a short pulse of *UFO* expression during early stages of flower development is sufficient to restore normal petals and stamens. From our results emerges a model of the different roles of *UFO* during flower development that will be presented.

5-08 Mutational analysis of the DNA binding domain of AINTEGUMENTA

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The Arabidopsis protein AINTEGUMENTA (ANT) is an important regulator of growth during flower development. ANT is a member of the AP2/EREBP family of plant-specific transcription factors that contain either one or two AP2 repeats. Although this is one of the largest families of transcription factors in Arabidopsis, relatively little is understood about how these proteins interact with DNA. The co-crystal structure of the single AP2 repeat of AtERF1 bound to DNA reveals that the protein uses a three-stranded b-sheet to make major groove contacts. To better understand the mechanism of gene regulation by proteins containing two AP2 repeats, we have utilized a genetic screen in yeast to identify amino acids that are critical for the DNA binding ability of ANT. This screen combined the inherent infidelity of taq polymerase and homologous recombination to generate and screen a large number of mutants containing alterations within the two AP2 repeats and linker region of ANT. Mutants that were no longer able to activate the expression of a reporter gene under the control of ANT binding sites were identified. A secondary Western screen eliminated those mutations resulting in premature termination, frameshifts, or grossly altered levels of expression. A number of the mutations identified do not map to residues predicted to contact DNA based on the AtERF1 structure. Several of the mutant proteins were expressed in E. coli and their DNA binding properties examined in vitro. The implication of these results for understanding ANT-DNA interactions will be described.

5-09 Isolation and characterization of genes involved in male fertility in *Arabidopsis*

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Male sterility is more and more used in hybrid breeding of oilseed rape crops. We are interested in using the genes involved in male fertility from *Arabidopsis* to improve the hybrid breeding systems for rapeseed production. We report here the identification and isolation of useful genes involved in sporophytic process of male fertility using gene-trap and enhancer-trap technologies (Sundaresan et al 1995: Gen & Dev 9: 1797-1810). So far, 67 male sterile mutants have been isolated and are being characterized. In mutant SET2640, the insertion of enhancer-trap *Ds* element into a transporter-like gene located on chromosome IV blocks the formation of pollen grains. In mutant anthers, the microspore mother cells undergo meiosis to give rise to microspores, but the microspores can not proceed to complete development and are degenerated when anthers are mature. Mutant anthers do not dehiscence, indicating the mutation might also affect anther wall development. No abnormality in other parts was observed on mutant plants. The female gametes are normal and fully fertile and the plant can get full seed setting when it is pollinated with wild type pollens.

5-10 Expression analysis of the *Arabidopsis* transcriptional regulator MALE STERILITY1

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The *MALE STERILITY1* (*MS1*) gene is critical for the production of viable pollen, since when mutated no pollen is produced and the plant is male sterile. Female fertility is however unaffected and the mutant can be rescued by fertilization with wild type pollen. In the ms1 mutant the process of pollen development begins normally, with meiosis and tetrad formation progressing as in the wild type. However, immediately after microspore release from the callose wall the immature pollen begins to breakdown. Around this stage the tapetal tissue also appears abnormally vacuolated.

We have cloned the *MS1* gene (Wilson et al. Plant J. 28:27-39) and found at DNA level that there is no particular homology to other sequences in the database, particularly those associated with microsporogenesis. However, the deduced MS1 protein has significant homology to the PHD-finger motif found in transcription factors from humans, yeast and higher plants. We therefore believe that *MS1* is a sporophytic controlling factor for anther and pollen development.

The *MS1* gene is expressed at low levels in immature flower buds, but no expression is seen in open flowers. Preliminary molecular analyses have shown that *MS1* appears to be expressed predominantly within the tapetal tissue around the stage of microspore release. This corresponds to the tissues and stage when phenotypic aberrations are seen. We have been conducting detailed RT-PCR analyses and *in situ* hybridisations to determine the exact timing and pattern of *MS1* gene expression. This data and the potential role of the *MS1* gene will be discussed.

5-11 Cotton *GhACT1* gene is preferentially expressed in fiber and required for fiber elongation

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Each fiber of cotton (*Gossypium hirsutum*) is a single epidermal cell that rapidly elongates to 2.5 - 3.0 cm from ovule surface within about 16 days after anthesis. A large number of genes are required for fiber differentiation and development, but it is unknown how these genes control and regulate the process of fiber development. To investigate the role of cytoskeleton during fiber development, GhACT1 cDNA, encoding actin protein, was isolated from a cotton fiber cDNA library. Northern analysis demonstrated that GhACT1 transcripts accumulated at high levels in fiber, and at very low levels in other tissues. Subsequently, the corresponding GhACT1 gene including its promoter was isolated, and the GhACT1 promoter was fused with GUS reporter gene. Histochemical assays in a large number of transgenic cotton plants showed that the GhACT1::GUS fusion gene was preferentially expressed at high levels in fiber, and at low levels in other tissues such as ovule, seedling cotyledon. To study the role of the GhACT1 gene during fiber development, anti-sense GhACT1 gene was introduced into cotton. Fiber cell elongation in transgenic plants was dramatically reduced, as a result of the reduction of actin proteins in fibers. The results suggested that the GhACT1 gene plays a role in fiber elongation but not fiber initiation.

5-12 Roles of *SEPALLATA* and *AGL3* genes in flower development

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During *Arabidopsis* flower development a set of MADS-box homeotic genes plays a central role in specifying the identities of the four whorls of organs. Among these are the *SEPALLATA* (*SEP*) genes which have recently been shown to play a key role in the identity of the three inner whorl organs. Triply mutant plants lacking the activities of all three *SEP* genes display a transformation of petals, stamens and carpels into sepals. This and other studies (Honma and Goto, 01; Pelaz et al., 00, 01) have given rise to the modification of the ABC model of flower organ identity. In this revised model, the MADS-box proteins involved in flower organ identity form distinct protein complexes for each of the four whorls. The formation of these complexes seems to be an indispensable requirement that results in the specific transcriptional regulation of the target genes. The *AGL3* MADS-box gene, which is very closely related to the *SEP* genes, is expressed in all aerial parts of the plant. We have found an *agl3* T-DNA insertion mutant, and although *agl3* single mutants appear similar to wild-type plants, the *agl3* mutation enhances the *sepallata* triple mutant phenotype. The quadruple *sep1/2/3 agl3* mutant flowers develop new flowers in the axils of the first whorl organs, similar to *ap1* mutants, and the floral organs have leaf features instead of sepal identity. The combination of *sep* and *agl3* mutants leads to the development of flowers with almost no “flower” identity.

5-13 Analysis of the capulet gametophytic maternal-effect mutants in *Arabidopsis thaliana*

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Maternal effects are fairly common in genetically tractable animals, such as *Drosophila melanogaster* and *Caenorhabditis elegans*. By contrast, the evidence for maternal effects in higher plants is rather scant, which is related to the plant-specific alternation of generations, with a haploid gametophyte being interspersed between two successive diploid sporophytic generations. After double fertilization, the female gametophyte of higher plants gives rise to the diploid embryo and the triploid endosperm which develop in concert to produce the mature seed. What roles gametophytic maternal factors play in this process is not clear. Here we describe novel gametophytic maternal-effect mutants in *Arabidopsis*. In the *capulet* (*cap*) mutants, both embryo and endosperm development is arrested at early stages. The *cap* mutant phenotypes were not rescued by wild-type pollen nor by pollen from tetraploid plants. Furthermore, removal of silencing barriers from the paternal genome by *METHYL TRANSFERASE1* antisense transgene expression or by mutation in the *DECREASE IN DNA METHYLATION1* (*DDM1*) gene also failed to restore seed development in the *cap* mutants. The mutants displayed no autonomous seed development. In addition, *cap2* was epistatic to *fertilisation-independent-seed1* (*fis1*) in both autonomous and sexual endosperm development. Finally, the paternally inactive endosperm-specific *FERTILISATION INDEPENDENT SEED2* (*FIS2*) promoter *GUS* fusion transgene was activated normally in *cap* endosperms. Molecular characterization of the *CAPULET* genes, their expression patterns and sub-cellular localization of their products is in progress, and will provide new insight in the mechanisms involved in embryo and endosperm development.

5-14 Identification of proteins that interact with INDEHISCENT, a bHLH transcription factor required for fruit dehiscence

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The Arabidopsis fruit releases its seeds through a process referred to as pod shatter or fruit dehiscence. Several genes involved in this process have been identified. One of these factors is a basic helix-loop-helix (bHLH) transcription factor called INDEHISCENT (IND). IND is necessary for the differentiation of the fruit dehiscence zone and for the lignification of the cell layer adjacent to the dehiscence zone.
To better understand its role in fruit dehiscence, we have initiated a yeast two-hybrid screen to identify proteins that may interact with IND. Current results will be presented.

5-15 *AGL11* an ovule specific gene in *Arabidopsis thaliana* functional analysis

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Most of the well characterised MADS box genes in plants are homeotic genes that play key roles in determining flower architecture. *AGL11* is a MADS box gene expressed specifically in the ovule of *Arabidopsis thaliana* (Rounsley et al 1995); its expression persists during seed development. It shares high similarity with the Petunia genes *FBP7* and *FBP11* that are involved in the determination of ovule identity (Colombo et al 1995), and with *OsMADS13* an ovule specific gene of rice (Lopes et al. 1999). The ectopic expression of *AGL11* in *Arabidopsis* resulted in the presence of curved rosette leaves and bracts and in the conversion of sepals into carpelloid organs that can develop mature ovules.
In order to identify genes that are regulated by *AGL11* we fused the rat glucocorticoid receptor (GR) to the gene to be able to induce *AGL11* activity in *Arabidopsis* by treatment with the dexamethasone hormone. By combining the dexamethasone treatment with the a protein synthesis inhibitor, direct targets of the *AGL11* gene can be isolated. We are planning to use Micro-arrays or cDNA-AFLP approaches (Sablowski et. al 1998). The first preliminary results will be presented.

-Rounsley S. D. et al 1995: Plant Cell 7:1259-1269
-Colombo L. et al 1995 : Plant Cell 7 : 597-604
-Sablowski R.W.M et al 1998 : Cell 92 : 93-103
-Lopes Z.P. et al. 1999: Developmental Genetics 25: 237-244

5-16 *AtD123* gene is alternatively spliced in *Arabidopsis thaliana*

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A gametophytic mutant was identified by screening *Ds* insertion lines. Segregation ratio of KanR to KanS in this mutant was 1:4, instead of typical Mendelian 3:1 segregation. Southern and sequencing data showed that a single *Ds* was inserted between two putative ORFs. Complementation experiments demonstrated that the upstream gene, *AtD123*, mainly affected the female development, while the downstream gene contributed to the male sterile phenotype. Eight cDNA clones were isolated from a flower cDNA library using an *AtD123* probe. Sequence analysis of the cDNA clones indicated that six clones encoded the same peptide, one clone was chimeric and one was a product of alternative splicing of a 205bp intron from the full- length clone, resulting in the truncation of one-third of the ORF at the C terminus. The splice junction is consistent with the highly conserved consensus GT-AT. In addition, these clones varied in poly(A) addition at their 3′;UTR. Alternative splicing was confirmed by RT-PCR: using a pair of primers flanking the start and stop codons respectively, two fragments, *AtD123L*(1002bp) and *AtD123S* (797bp), were amplified from mRNAs isolated from all tissues in *Arabidopsis*. However, the ratio of the two transcripts differed in various tissues: 6.4 : 1 in seedling, stem, leaf, and flower, and 12.8 : 1 in root and silique. The functional significance of *AtD123* alternative splicing during gametogenesis is being investigated.

5-17 A high affinity amino acid transporter in developing seeds of Arabidopsis

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The developing seed depends on the transporter-mediated import of nutrients, ions, amino acid and sugars to synthesize storage proteins and polysaccharides. The import systems for amino acids must cover with their affinities the whole range of available substrates to be able to import all substrates during seed development and to adapt to varying supply. A new member of the amino acid permease family, AAP8 was identified in the Arabidopsis genome and isolated by RT-PCR. Functional characterization by heterologous expression in a yeast mutant deficient in several amino acid uptake systems showed that AAP8 transports a wide spectrum of amino acids. Remarkably, AAP8 mediated more efficient growth on aspartate as sole nitrogen source compared to the already characterized high affinity transporter AAP6. Determination of the KM value for aspartate for AAP8 by uptake experiments confirmed a high affinity and capacity for aspartate uptake. Expression analysis revealed that AAP8 is expressed in a stage-dependent profile in the vasculature of siliques and in developing seeds. The expression pattern differs from other AAPs that are expressed in developing siliques. AAP1 is expressed exclusively in seeds and AAP2 exclusively in the vascular tissue of the silique. These two proteins have different affinities for amino acids compared to AAP8. Especially aspartate is transported by AAP8 with a higher affinity and capacity. The biochemical properties of AAP 8 and its expression in both seeds and vascular strands make this gene an ideal candidate for completing the import system of amino acids into developing seeds. Thus, although AAP1 and AAP8 are located in a duplicated region on chromosome 1, none of the AAPs seems to be a product of simple gene duplication or redundancy.

5-18 Cell polarity and cell fate determination during female gametogenesis in Arabidopsis

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Alternation between a sporophytic, diploid phase and a gametophytic, haploid phase is a fundamental process evolved to maintain genetic stability and eliminates lethal mutations through meiosis in eukaryotes. In flowering plants, the female meiocyte undergoes meiosis to give rise to four haploid megaspores among which one, two or all four spores may participate in the formation of the female gametopyte, the embryo sac, respectively in different plant species. Majority of higher plants are monosporic and belong to Polygonum (>70%) and Oenothera type. In the Polygonum type like Arabidopsis, only the chalazal megaspore develops into an embryo sac, whereas the micropylar three megaspores degenerate. In contrast, the micropylar megaspore is functional in Oenothera type. Why do the megaspores, though originated from the same meiocyte, undertake different cell fate? What is the determining factor there? To answer these questions, we have performed a genetic screen for gametophytic mutations and studied the role of polarity during female gametogenesis. One mutation that affects megaspore cell fate resulted in ovule abortion. The aborted ovules are arrested at different developmental stages. More intriguingly, in about 5% of the aborted ovules, instead of a single embryo sac there were four large cells varying in size. These cells are from tetrad, suggesting that three micropylar megaspores fail to undergo apoptosis, but survive and acquire the ability to form embryo sacs and express a synergid-specific marker gene. The molecular characterization of the mutation is being carried out.

5-19 Control of *UFO* expression

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The *UNUSUAL FLORAL ORGANS (UFO)* gene is expressed in all shoot and floral meristems. Using two different approaches – a promoter deletion analysis and a mutagenesis screen based on changes of the *UFO* expression pattern – we intend to identify factors that are involved in control of *UFO* expression. Analysis of transgenic plants carrying *UFO* promoter fragments fused to a *GUS* reporter gene has revealed the identity of seperate elements that control shoot and floral expression of *UFO*. We are currently performing yeast one-hybrid screens to identify factors that bind to such elements. Analysis of EMS-mutagenized homozygous *UFO::GFP* lines at the seedling stage led to the identification of several candidates that might play a role in regulation of *UFO* expression.

5-20 Analysis of a male gametophytic Arabidopsis mutant affected in the progamic phase

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Pollen-pistil interactions in plant reproduction involve pollen deposition onto the stigma, pollen germination, tube growth and guidance of the pollen tube by the pistil, leading to delivery of the sperm cells to the embryo sac. Pollen tube growth and signalling between pollen tube and pistil together are referred to as the progamic phase. One approach to study genes involved in this unique intercellular process is screening *Arabidopsis* T-DNA or transposon-tagged populations and search for lines with a non-mendelian segregation of the antibiotic resistance gene, due to reduced T-DNA transmission (Howden *et al,* Genetics 149, 621).
The Arabidopsis line TJ995, of the enhancer trap population generated by Dr. T. Jack (http://www.dartmouth.edu/~tjack/), shows strongly reduced male transmission (2%), whereas female transmission of the T-DNA is unaffected (100%). Pollen and seed development appear normal, as does pollen germination *in vitro*. This suggests the insertional inactivation of a gene that is expressed in the male gametophyte and is involved in the progamic phase. Multiple T-DNAs are present within the genome of TJ995 and they are organized in a complex manner. Although complete analysis of the insertion sites is still in progress, TAIL-PCR analysis shows that a T-DNA is inserted within a hypothetical gene, positioned on BAC T32M21. This gene is expressed in pollen. Data on complementation and on analysis of an independent T-DNA insertion line will be presented, elucidating the involvement of this gene in the *Arabidopsis* progamic phase.

5-21 *PsNIP-1*, the pea orthologue of soybean Nodulin 26, is expressed in developing seed coats and downregulated by phosphorylation

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The family of Major Intrinsic Proteins (MIPs) consists of pore-forming proteins that facilitate the trans-membrane flux of water and/or small neutral solutes like glycerol. Plant MIPs include the plasma-membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), and the nodulin 26-like intrinsic proteins (NIPs). Nodulin 26 is an abundant protein in the peribacteroid membrane of soybean root nodules.
To study the role of MIPs in seed coat unloading, we recently cloned four MIP-genes from a cDNA bank of coats of developing pea seeds. One of these, PsNIP-1, is apparently the orthologue of Nodulin 26 as it was also expressed in root nodules. Heterologous expression in *Xenopus* oocytes demonstrated the permeability of PsNIP-1 for water and glycerol, which could be downregulated by cAMP-dependent phosphorylation. Site-directed mutants are now being generated to pinpoint the residues involved in phosphoregulation, and antibodies raised against Nodulin 26 will be used to assess the expression of PsNIP-1 at the (sub)-cellular level.

5-22 Module-specific expression from the phas promoter during embryogenesis in transgenic Arabidopsis

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The strict spatial regulation of transcription from *b-phas*, which encodes the major seed storage protein of French bean, *Phaseolus vulgaris* was shown to be maintained in Arabidopsis, as was previously established in tobacco and other transgenic plants. Dimethyl sulfate (DMS) *in vivo* footprinting analyses revealed that some twenty three *cis*-elements within the proximal 295 bp of the phas promoter are protected by factor binding in seed tissues whereas none are bound in leaves. The possibility that the complex footprinting profile represents a summation of several module-specific (cotyledon, hypocotyl and radicle) factor-DNA interactions has been examined by incorporating site-directed substitution mutations into the *-295phas* promoter and evaluation of reporter (GUS) expression intensity and pattern in transgenic Arabidopsis. Both redundancy and hierarchy was detected in cis-element functions.The G-box and CCAAAT box were identified as major positive elements, and the E-box and RY sites were shown to function in mediating high levels of expression in seeds. The G-box, but not the ACGT motif, may be the functional abscisic acid responsive element. The CACA element has dual positive and negative regulatory roles and the vicilin box is a bona fide repressive element. Elements important in modulating radicle-specific expression include RY elements at positions -277 to -271; -260 to -254 and -237 to -231. The proximal (-70 to -64) RY motif contributes strongly to expression in the hypocotyl while all the RY elements contribute to expression from the phas promoter in cotyledons but not the vascular tissues during embryogenesis.

5-23 Analysis of the role of *UFO* in flower development by identifying its interacting factors

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UNUSUAL FLORAL ORGANS (UFO) is a positive regulator of *APETALA3 (AP3)* expression. In addition to this regulatory role, *UFO* also has additional roles in flower development, such as defining boundaries within floral primordia and controlling cell proliferation. *UFO* encodes a protein containing an F-box, which was shown to be responsible for the interaction with Arabidopsis Skp1 like proteins, ASK1 and ASK2*. Many F-box proteins are known to be components of a functional SCF complex (Skp1, Cullin, and F-box), a ubiquitin protein ligase (E3) that brings substrates to the complex and thereby targets them for degradation by the proteasome. However, some of F-box proteins form non-SCF complexes with a variety of proteins in vivo. To define the role of *UFO*, we are exploiting two different strategies: yeast two-hybrid screens and immunopurification of a protein complex including UFO.
In our yeast two-hybrid screen, DFUFO, which has a deletion in the F-box, was used as bait to facilitate the interaction with factors other than ASK1 and ASK2. A cDNA library from Arabidopsis inflorescences was screened for interactions. 4.5 million clones were screened and 37 candidates recovered. Over a half of the candidates (19/37) encode ubiquitin conjugating enzymes (E2s), suggesting that UFO has a role in targeting substrates for ubiquitination. In order to circumvent limitations of the yeast two-hybrid screen, we are using transgenic plants containing a 35S-myc tagged UFO construct to immunopurify a protein complex containing UFO. Progress in identifying in vivo factors that copurify with UFO will be presented.

-Samach A, et al, 1999, Plant Journal 20, 433-445

5-24 The role of the *REPLUMLESS* (*RPL*) gene in fruit development

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During the formation of the fruit, several specialized tissues differentiate to protect the developing seeds and subsequently disperse the mature seeds. In *Arabidopsis*, the fruit consists of valves, or pod walls, a central replum and septum, and the valve margins that attach the valves to the replum and later separate to release the mature seeds. The *SHATTERPROOF* (*SHP*) MADS domain transcription factors and the *INDEHISCENT* (*IND*) and *ALCATRAZ* (*ALC*) bHLH transcription factors all are involved in specifying valve margin fate and control the opening of the fruit. The *FRUITFULL* (*FUL*) MADS domain transcription factor specifies valve identity at least in part by repressing the expression of valve margin genes. However, factors involved in specifying the replum have been a mystery. The *ful* mutant has an enlarged, twisted replum that is easily visible and can be used to more easily identify mutations that affect replum development. In a mutagenesis of *ful* plants, a mutant with no visible replum was recovered. In this *replumless* (*rpl*; pronounced "ripple") *ful* double mutant, the replum cells are replaced with cells that resemble the *ful* "valve" cells, which have partial valve margin identity. Likewise, single *rpl* mutant replum cells appear similar to valve margin cells. Only the outer layers of the *rpl* replum are affected. Markers for the valve margin including *SHP2::GUS* and GT140 are expressed in the outer replum cells of the *rpl* mutant suggesting that the *rpl* gene is involved in specifying replum cell fate by repressing the expression of valve margin genes.

5-25 Characterization of the early flowering mutants, *fsu2*, obtained from activation-tagging mutagenesis in *Arabidopsis thaliana*

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

5-26 Molecular genetic analysis of the *magatama3 (maa3)* mutant

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Sexual reproduction in plants, unlike that of animals, requires the action of haploid gametophytes. We have taken a molecular genetic approach to study the development of female gametophyte. To isolate female-gametophytic mutants, we looked for lines in which half of the seeds do not develop. This procedure is based on the assumption that, in a heterozygote of the female-gametophytic lethal line, half of the female gametophytes inherit the mutant allele and cannot make seeds. We isolated four mutants which we named *magatama (maa)* (Shimizu and Okada, Development 127, p. 4511-8, 2000). Here we will describe the molecular genetic analysis of *maa3* Microscopic observation of cleared female gametophyte revealed two defects. First, the development of the *maa3* female gametophyte was slower than that of wild-type. At anthesis, the fusion of two polar nuclei had not finished yet. Second, the size of the nucleoli was small. In addition, pollen tube guidance by female gametophyte was defective.
maa3 mutation was tightly liked to a T-DNA. Using inverse PCR, we found that the T-DNA disrupted a large gene with 21 exons. The *MAA3* gene encodes a protein with RNA helicase motif, which is known to be involved in the maturation of rRNA or small nucleolar RNA. It is suggested that the dysfunction of the helicase causes the defect of the nucleolus and/or ribosome, and slow growth.

5-27 Exine patterning is disrupted by reduced callose accumulation in *kompeito*

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The first step of cell-cell interaction between male and female reproductive organs is the pollen-stigma adhesion, in which pollen grains are physically attached to the stigma before hydration. We previously showed that the morphology of the outermost layer of the pollen grains, the exine layer, is important for this adhesion step, using *kompeito* mutant that is defective in the exine sculpture. However, little is known about the formation of the exine sculpture. TEM analysis revealed that, in addition to the exine sculpture, *kom* was defective in callose accumulation during microsporogenesis. To observe the callose wall precisely, we made a series of sections of floral buds and stained with aniline blue. In *kom*, no callosic staining was found during meiosis stage. In tetrad stage, callose wall was observed, but the amount of callose was much smaller than wild type. At the same stage, sporopollenin was randomly deposited onto the plasma membrane of the microspore, and following exine formation was disrupted. We previously isolated the *KOM* gene by map-based cloning and it encoded a seven-path transmembrane protein. *In situ* hybridization analysis showed that *KOM* was expressed in pollen mother cell during meiosis stage. No *KOM* expression was observed before or after meiosis. These results suggested that *KOM* is essential for the meiosis stage-specific callose accumulation.
Based on our results, it is suggested that callose wall is important for the formation of the exine layer, and that *KOM* may regulate the callose synthesis.

5-28 Isolation and analysis of direct target genes for the transcriptional regulation of *ATHB-1*

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ATHB-1 is a member of HD-Zip-type transcription factors. Its molecular functions, e.g., sequence-specific DNA binding and transcriptional activation, have been studied in detail so far. However, it is still unclear what processes concern ATHB-1 during plant development and how they are regulated by ATHB-1. To address these questions, direct target genes of ATHB-1 were isolated and the manners of their transcriptional regulation were analyzed. We have constructed a modified ATHB-1 of which transactivating function is steroid-inducible in transgenic *Arabidopsis* plants. For genes inducibly transcribed by the modified transcription factor, we screened a cDNA microarray containing about 2200 independent clones, and selected 34 positive clones as candidates for direct target genes of ATHB-1. Each gene was examined for its direct activation by the modified transcription factor in a Northern analysis using RNA preparations from plants treated with dexamethasone and/or cycloheximide, and for the correlation of its expression pattern with that of *ATHB*-1 in a histochemical analysis using transgenic promoter-GUS plants. Obtained direct target genes and their expression patterns will contribute to discuss the regulatory role of ATHB-1 during plant development.

5-29 Podshatter resistance: exploitation of Arabidopsis genes to develop a productivity trait in oilseed rape

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Plants have developed several fruit structures to propagate themselves through the distribution of seeds. Arabidopsis and Brassicas form pods or siliques which release the seed following the separation of the valves. This active process during fruit maturation is called dehiscence. In crop plants where seeds are the harvested product, this developmental process can have a big impact on seed recovery and hence yield.
Recently, several genes, which play a regulatory role in pod dehiscence have been identified in Arabidopsis through mutant analysis. The genes are specifically expressed in pods and either promote or inhibit dehiscence: The scope of this work is to define the role of these genes in pod development using them as tools to control valve separation in Arabidopsis and shatter control in oilseed rape (Brassica napus).
Shedding of the seed before and during crop harvest represents an inherent problem in Brassica napus. We have demonstrated that seed shatter can be controlled through the inhibition of the expression and/or overexpression of specific regulatory genes in B. napus.

5-30 Analysis of T-DNA insertion mutants affected in fruit development

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Fruit development is a process of interest from both basic and applied research perspectives. The *Arabidopsis* fruit is a typical silique, just like those of other members of the *Brassicaceae*. Given the properties making *Arabidopsis* an excellent model organism for the genetic and molecular analysis of developmental processes, we expect that the study of fruit development in this model plant will provide new insights on the mechanisms involved in making the fruits, as well as the proposal of models to explain fruit morphogenesis in other plant species. To help understand this developmental program in *Arabidopsis*, we have undertaken a screening for mutants affected in fruit shape or size in a T-DNA-mutagenized population of *Arabidopsis*. A number of mutants have been isolated which show abnormal fruit morphology. The *pepper* (*pep*) mutant shows extra number of carpels that fuse into a silique, and enlarged replum. The recessive mutation responsible for the phenotype cosegregated with the insertion, allowing the cloning of a plant DNA sequence adjacent to the T-DNA and the identification of the corresponding gene. *PEP* codes for an RNA binding protein with KH domains, a gene class known to play important roles in animal development. Other mutant, *fup* (*fructus parvus*, the latin for small fruit), displays fruits with a rough surface, and abnormal abaxial replum and mesocarp. Although the insertion in this mutant also cosegregates with the mutation, the cloning of an adjacent genomic *Arabidopsis* DNA did not reveal the presence of a gene tagged by or in the proximity of the T-DNA. An accurate mapping of the mutation will be presented for discussion.

5-31 Molecular characterisation of the Self-Incompatibility (SI) response in Crucifers

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Plant have mecanisms to promote outbreeding and thereby to increase their genetic diversity. In self-incompatible crucifers, recognition and rejection of the self-pollen is based on a receptor-ligand interaction between male and female SI determinants. A transmembrane receptor-kinase (S-locus Receptor Kinase, SRK) determines the SI specificity in stigma cells whereas a pollen coat-localized ligand (S-locus Cystein Rich protein, SCR) determines the SI specificity in pollen. During the past years, major advances have been made in the understanding of the recognition phase of the SI. The next challenge will focus on identifying components of the SRK-triggered signaling cascade and understanding how the stigma cells can locally prevent pollen germination. In order to identify genes involved in this pahway, we are developping molecular approachs using *A. lyrata*, an outcrossing close relative of the self-fertile *A thaliana*. The high degree of sequence similarity between both *Arabidopsis* species allow easy transfer of genetic markers and other molecular tools generated by the *A. thaliana* genome project to *A. lyrata*. Moreover, SI response in *A. lyrata* is similar to that of other SI crucifers, in its physiology as well as in its molecular control. These features make *A. lyrata* an attractive model to study further, the molecular basis of self-pollen rejection.

5-32 On flowers and cones: Comparative analyses of developmental control mechanisms in Arabidopsis and the conifer Norway spruce

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In Arabidopsis, flower development requires the activity of the flower meristem-identity gene *LEAFY*, which activates the flower organ-identity genes, many of which belong to the MADS-box gene family. We have shown that genes homologous to LEAFY and to a subset of the flower organ-identity MADS-box genes are present in the conifer Norway spruce and are active in developing seed cones and pollen cones. Genes homologous to the B-genes which regulate stamen and petal development in angiosperms are active specifically in developing pollen cones in spruce, and genes homologous to the C-genes, required for stamen and carpel development, are expressed exclusively during pollen- and seed-cones. In itself, this indicates conservation of theses regulatory mechanisms for development of the reproductive organs, among all seed plants. A functional conservation between the homologous conifer and angiosperm proteins is supported by data on the effects of constitutive expression in Arabidopsis of the conifer genes, for both B- and C-type genes. In addition, we have also identified MADS-box genes that are potentially unique to either the conifers or the angiosperms, and which are active in developing reproductive organs. These may have roles relating to the evolution of different morphologies found in the reproductive structures of the different groups of seed plants.

5-33 A female gametophytic mutant in *Arabidopsis thaliana* affected in the interaction between pollen tube and embryo sac

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In flowering plants, a prelude to fertilization and subsequent seed development is the interaction between the two gametophytes: the pollen tube and the embryo sac. This interaction, which involves two haploid organisms, is unique and interesting, but still not well understood. How does the pollen tube enter the synergid cell and discharge its cytoplasmic content, including the two male gametes? What is the role of synergid cell degeneration? No genes directly implicated in fertilization steps have been identified so far.
To identify such genes, after gamma-ray mutagenesis of *Arabidopsis thaliana* seeds, we screened plants for normal gametophytes but fertilization defect, as seen at cellular level. In the present work, we describe one of the mutant lines obtained, which is affected in the pollen tube / embryo sac interaction. The phenotype of this mutant was characterised using D.I.C and confocal-laser microscopy. In the gametic complex of mutant embryo sacs, pollen tube overgrows, makes turns, and does not discharge its content preventing fertilization. Genetic evidences show that this mutation exhibits a female gametophytic phenotype. A line, obtained from A. Cheung (Amherst, MA, USA), that express GFP in the vegetative cell of the pollen tube was used as male parent in crosses with our mutant line. In order to monitor, in these crosses, the final growth of the pollen tube from the micropyle to the synergid cell, we have designed a semi-vitro system compatible with confocal-laser microscopy. Precise phenotypic description obtained with this imaging system will be presented.

5-34 *NOZZLE* (*NZZ*) interacts with a member of the *YABBY* gene family in yeast and *in-vitro*

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Arabidopsis plants homozygous for a *nzz* mutant allele exhibit sporophytic male and female sterility. Genetic and cytological data strongly imply a function for *NZZ* in patterning the developing ovule along the proximal distal (P-D) axis (Balasubramanian and Schneitz, 2000). *NZZ* is identical to *SPOROCYTELESS (SPL)* and encodes a novel protein with limited homologies to transcription factors (Schiefthaler et al., 1999; Yang et al., 1999). In a yeast-two-hybrid screen three bait-dependent interacting clones were found corresponding to two different genes. One candidate encodes a member of the *YABBY* gene family. *In-vitro* pull-down experiments with bacterially expressed proteins confirmed the interaction observed in yeast and were used to narrow down an interaction domain. *YABBY* genes are promoting abaxial cell fate in various above ground organs in *Arabidopsis* (Bowman, 2000). According to the principle of “guilt by association”, in which two interacting proteins are likely to participate in the same cellular function, this would implicate a role for *NZZ* not only in P-D but also in adaxial-abaxial (ad-ab) patterning. Further experiments to support the biological significance of the NZZ-YABBY interaction are undertaken and the results are shown.

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5-35 Isolation and initial characterization of *D18*, a putative positive regulator of *AP3* early expression

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In order to identify novel factors that control the onset and early expression of the Arabidopsis *APETALA 3 (AP3)* homeotic gene, we performed ayeast one-hybrid screen. We have isolated a putative transcription factor that includes a single Myb domain (*D18*), that specifically binds to the distal early element (DEE) of the *AP3* promoter. D18 is most similar to the Arabidopsis TELOMERE-REPEAT BINDING PROTEIN 1 (TRP1). D18-binding to the DEE element was demonstrated in vivo by yeast *LacZ* assays, and in vitro by gel shifts. In the latter, we have found that D18 binds to a palindromic sequence closely situated to the LFY-binding site in the DEE element of the *AP3* promoter.
In addition, to test the possible functional role of *D18* in planta, we have generated *D18* antisense-inducible transgenic plants, in which the loss of *D18* expression was induced by dexamethasone(DEX) treatment. We obtained several homozygous lines for the transgene and examined their phenotype after 10 days of 10 uM DEX treatment. We observed abnormal flower phenotypes such as reduced growth in petals and sepals, and defects in internode elongation. In some less severe lines, flowers with a reduced stamen number (four or five in the antisense lines versus six in the wild type plants) were more frequently found in DEX treated plants than in ethanol treated control. Finally, Northern Hybridization showed that *AP3* expression was reduced in the antisense lines. These results suggest that D18 positively regulates *AP3* transcription and may do so by specifically binding to the DEE element of the *AP3* promoter.

5-36 Tracking cell-cell interactions during the reproductive proccess in *Arabidopsis thaliana*

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Complex signalling events between female tissues and male gametophytes represent the major interplay to overcome several checkpoints established by the female cells. This leads to the correct guidance of pollen tubes to their ultimate target, the embryo sac. We carried out a forward genetic screen in *Arabidopsis thaliana* to identify mutants with defective interactions during the reproductive process and affecting mainly the male gametophyte. We describe a mutant where the primary recognition contact between pollen and stigma is disrupted. Pollen adhesion to stigmatic cells and subsequent steps of hydration and germination are inhibited. Mutations affecting one or both gametophytes showing altered responses to guidance signals are described. We report a technical advance using two-photon microscopy on living tissues. Using a LAT52::GFP expressing line, a rapid tracking of pollen tube behaviour *in vivo* and in intact flowers allows visual examination in real time of early steps of hydration, pollen tube growth, and guidance. This technique represents a straight approach to *in vivo* analysis of fundamental mechanisms underlying cell-cell interactions or mutations that disrupt pollen function.

5-37 Transcriptional profiling of Arabidopsis pollen and pollen tubes by high-density oligonucleotide arrays

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Pollen is the male gametophyte of higher plants thus having a crucial role in the process of sexual reproduction. Upon hydration on the stigma pollen grains germinate and generate a tubular extension of the cytoplasm with unique growth features. Being one of the fastest growing cells in nature pollen tubes can reach a growing speed of several millimeters per hour. Their easy maintenance and in vitro manipulation make them one of the preferred experimental models to study tip growth of cells (Feijó et al., 2001).
Several studies have proven a close relationship between ion fluxes, namely calcium, protons, potassium and chloride, and the growth of pollen tubes. However, the genes encoding the proteins mediating these ion fluxes in pollen still have to be identified and their transcriptional profiles await characterization. Thus we are interested in establishing the genetic basis behind the control of ion dynamics in growing pollen tubes.
As a prerequisite for high quality data we have developed a method to isolate and purify *Arabidopsis thaliana* pollen by cell sorting. In order to identify the complete set of pollen- and pollen tube specific genes in *Arabidopsis*, we intend to compare the transcriptional profiles of pollen and pollen tubes to those of other *Arabidopsis* tissues by high-density oligonucleotide arrays. For a first approach we are using the "Affymetrix GeneChip Arabidopsis Genome Array" including over 8200 genes. Progress on the ongoing work will be presented.

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5-38 The homeobox gene *BREVIPEDICELLUS* is required for the normal development of style in Arabidopsis

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The mature gynoecium of *Arabidopsis* has four distinct pattern elements along its apical - basal axis: the stigma located apically, followed by the style, ovary and the gynophore, the basal most element. The ovary is made up of two congenitally fused carpels whereas the style and the stigmatic papillae are postgenitally fused structures. Ontogeny of the wild type gynoecium shows that at stage 7, the gynoecial primordium is an open-ended cylinder that has distinct lateral and medial planes. Between stages 7 and 9 the stigmatic papillae and the style cells first differentiate along the medial plane closely followed by differentiation along the lateral plane. By stage 9, the style, stigma and the valves of the ovary become distinct from each other. *CRC* and *SPT* genes are critical for the differentiation of style cells in the medial plane, mutations in either gene leads to loss of postgenital fusion of the septum. The *brevipedicellus* (*bp*) mutant is defective in cell differentiation in the lateral plane of the style and by stage 9 reveals a distinct asymmetry. Our recent studies showed that *BP* gene encodes the homeodomain protein KNAT1 and regulates inflorescence architecture (PNAS 99:4730-5. 2002). The results on the role of *BP* in the establishment of the cylindrical style, one of the radially symmetrical elements of the gynoecium will be presented.

5-39 *NEVERSHED* controls floral abscission in *Arabidopsis*

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After pollination, many flowers shed their outer organs through the developmentally and hormonally regulated process known as abscission. Pioneering studies over the past few decades have demonstrated important roles for the plant hormones ethylene and auxin in regulating abscission, and have also identified some of the hydrolytic enzymes involved in the separation process. However, the molecular circuitry controlling abscission zone differentiation, the hormone signaling that regulates the timing of separation, and the secretory machinery involved in the separation process itself are largely unknown. Through EMS mutagenesis screens of adult *Arabidopsis* plants, we have identified five independent alleles of a recessive mutant in which shedding of the sepals, petals and stamens fails to occur throughout the plant life cycle. Thus far, our analyses of the *nevershed* mutant and the corresponding gene suggest that *NEVERSHED* may represent an integral component of both the developmental and hormonal pathways regulating floral abscission.

5-40 *ETTIN* and gynoecium development

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In Arabidopsis, the female reproductive organ, or gynoecium, consists of distinct tissue types: an apical stigma, style, and basal ovary. *Ettin* (*ett*) mutants affect the patterning of these different tissue types and display an allele-strength-dependent loss of valve tissue within the ovary. ETT is a member of the ARF (auxin response factor) family of transcription factors. To further study the role of ETT in regional patterning of the gynoecium, an activation tagging modifier screen was performed in a weak *ett-2* geneticbackground. One enhancer mutant line, 1771, displayed a decrease in valve number and valve size. Using TAIL PCR and Southern blot analysis, two genetically linked T-DNA were mapped to chromosome I. The right T-DNA border contains enhancer elements (1) which when inserted near a gene may enhance its transcription. A putative *TFL/FT* gene is located 300 bp adjacent to the right T-DNA border. Its mRNA abundance was shown to be increased in line 1771 when compared to *ett-2* mutants. Attempts to recapitulate the enhanced *ett* phenotype by expressing a 35S::TFL/FT construct in *ett-2* mutants are underway. This may elucidate a potential role for TFL/FT in the ETTIN signalling pathway.

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5-41 TFL1 protein trafficking is necessary for the coordinated differentiation of the inflorescence meristem

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The *TERMINAL FLOWER 1 (TFL1)*gene of *Arabidopsis* serves a key function in the development and differentiation of the inflorescence meristem. Using GFP fusions, we have shown that TFL1 protein moves from the inner region of L3, where the *TFL1* RNA is accumulated, to outer layers and this protein trafficking is regulated by the developmental phase of shoot apical meristems. We have also shown that L1 specific expression of TFL1 is sufficient to show phenotypes similar to those of the 35S::TFL1 plants, and which is due to the transmission of TFL1 protein from L1 to inner layers.
In order to inhibit TFL1 protein trafficking, we made transgenic plants expressing TFL1 proteins fused to two or three tandem GFPs. We found that the extent of 2GFP:TFL1 movement is lower than that of 1GFP:TFL1 and that 3GFP:TFL1 never moves. All of these proteins retain TFL1 function since plants expressing them by the 35S promoter show the same phenotype as 35S::TFL1 plants. While L1 specific expression of *1GFP:TFL1* (ML1::1GFP:TFL1 plant) shows similar phenotype of 35S::1GFP:TFL1 plants, ML1::2GFP:TFL1 or ML1::3FP:TFL1 plants show less severe phenotype. We are now making plants expressing 2GFP:TFL1 and 3GFP:TFL1 under the genomic context and phenotypes of these plants will tell us in which layer the TFL1 function is required. Currently, we are examining the movability of mutant or dissected TFL1 proteins. These results will be presented.

5-42 Understanding the regulation of flowering time: Characterisation of *fca-1* suppressor mutants

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FCA functions within the autonomous floral promotion pathway. *fca-1* plants flower late independent of day length, a phenotype that can be corrected by vernalization. This late flowering is due to increased *FLC* mRNA levels. To identify interacting pathways and genes downstream of *FCA*, a suppressor mutagenesis of *fca-1* was undertaken. We have been characterizing ten of these *fca-1* suppressor mutants (called *acf*). Genetic, physiological and molecular experiments have been carried out to determine why the different *acf* mutants flower early. Recent results using quantitative real-time RT-PCR are providing insights into the cause of the early flowering phenotypes. For example, the phenotype of *acf28 fca-1* plants, which in addition to flowering early has a small cabbage like appearance. This phenotype is probably largely the result of the upregulation of gibberellin genes. In comparison the early flowering of mutants, such as *acf32 fca-1*, correlates with a lower level of *FLC* mRNA. The results of these experiments will be presented as well as progress in the map-based of *acf32*.

5-43 Identification of AG targets during early stages of stamen and carpel development

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The morphogenesis of individual flowers is a genetically tractable model for pattern formation in plants. Based on genetic studies, the ABC model describes how three classes of homeotic genes act in discrete domains to specify the identity of floral organ types. All ABC genes encode transcription factors (in most cases from the MADS box family) so it is likely that they control sets of target genes required to form each type of organ. We have focussed on the C-function gene *AGAMOUS (AG)* that has previously been shown to be required either alone or in combination with the B-function genes *APETALA3 / PISTILLATA (AP3 / PI)* to direct carpel and stamen formation. We generated an inducible AG system by making a fusion protein with the glucocorticoid receptor (GR) domain. In this system the *ag-3* mutant was complemented in a dexamethasone (dex)-dependent manner. Rescue of reproductive organs by AG-GR however, only occurs when dex is applied at very early stages of organogenesis. To maximise the amount of early organs, 35S::AG-GR-containing plants were crossed into background homozygous for the *ap1 and cal1* mutations that cause the cauliflower phenotype. Steroid treatment caused semi-synchronous initiation of carpels and stamens, which developed into fully functional organs. We are currently using this system to identify changes in mRNA population after activation of AG-GR using cDNA arrays. Quantitative RT-PCR and *in situ* hybridization are being used to validate preliminary results.

5-44 *APS*, an Arabidopsis SBP-box gene affecting sporogenesis

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In Arabidopsis, all members of the structurally heterogeneous *SPL* gene family share a highly conserved and plant specific DNA-binding domain, the SBP-domain. Although likely to represent transcription factors, little is known about their role in development. Screening large transposon-mutagenised populations of Arabidopsis plants allowed the isolation of mutant alleles for one of the Arabidopsis *SPL* genes. In homozygous condition, these mutant alleles all resulted in a strong reduction in fertility primarily due to abnormally developed microsporangia. We renamed the corresponding *SPL* gene to *APS* for *ABERRANT POLLEN SAC*.

5-45 Identification and characterisation of mutants involved in meiosis in *Arabidopsis thaliana*

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Meiosis has been extensively described in plants. However, only a few genes involved in this process have been identified and characterised. *Arabidopsis thaliana* has been chosen as a model organism to study meiosis at several levels: genetics, cytology and molecular biology. The collection of *Arabidopsis thaliana* mutants of Versailles is a fantastic tool to tackle this project. A systematic screen for meiotic mutants has been performed on this material since 1993. The results obtained up to now will be presented. We will also present the characterisation of two genes, AtSWI1 and AtSPO11-1 identified through this screen. In parallel, we also performed reverse genetics to identify homologs of genes characterised in other model organisms and involved in meiosis.

5-46 *TETRASPORE* encodes a kinesin required for male meiotic cytokinesis in *Arabidopsis*

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In flowering plants, male meiosis occurs in the microsporocyte to produce four microspores, each of which develops into a pollen grain. Male meiosis is followed by a unique type of cytokinesis involving radial arrays of microtubules (MTs) which partition the cytoplasm into spore domains (Brown and Lemmon, 1988, *Amer. J. Bot*, 75: 1848-56). In *tetraspore* (tes) mutants of *Arabidopsis*, male meiotic cytokinesis is defective, and all four microspores begin development in a common cytoplasm, giving rise to large pollen grains which often contain extra and polyploid nuclei (Spielman et al., 1997, *Development* 124: 2645-57). We have found that *TETRASPORE* encodes a protein with an N-terminal kinesin motor domain, and an unusual C-terminal region with similarities to a small number of other plant kinesins. Immunolocalization studies show that *tes* mutants fail to form the radial MT arrays associated with microsporocyte cytokinesis. Therefore the *TES* gene encodes a kinesin required to establish spore domains following male meiotic cytokinesis.

5-47 *ESP*- an putative leucine-rich receptor kinase involved in anther development

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Arabidopsis anthers comprise four locules. Each locule is composed of a central core of sporogenous cells which enter the meiotic pathway giving rise to the male gametophyte (the pollen grains), invested by four defined anther wall layers: epidermis, endothecium, middle cell layer and tapetum. The innermost of these layers is the tapetum, which plays a number of key roles in pollen development. *ESP (EXTRA SPOROGENOUS CELLS)* function is critical in early stages of anther differentiation. Plants homozygous for the *esp* mutation lack an identifiable tapetum, have a variable number of anther wall layers and possess an increased number of sporogenous cells. Strikingly, commitment to meiosis in *esp/esp* plants is not affected by the absence of a tapetum layer, and chromosome pairing, condensation and division proceed normally. However, degeneration of the sporogenous cells occurs before completion of meiosis II ESP was cloned by positional mapping and found to encode a putative leucine-rich receptor kinase. Expression data will be presented.

6-01 SGT1, a RAR1 interactor, is an essential component of R-gene specific resistance

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Plant disease resistance (R) genes trigger innate immunity responses upon pathogen attack. Previous studies identified RAR1 as convergence point acting early in a signaling pathway engaged by multiple R genes in both barley and Arabidopsis. To investigate the mechanism of RAR1 function(s) in plants, we searched for interacting proteins by yeast two-hybrid screens using Arabidopsis RAR1 (AtRAR1) as bait. Two interacting proteins were identified sharing extensive amino acid similarity to each other (87%) and to yeast Sgt1. We designated the two Arabidopsis proteins AtSGT1a and AtSGT1b. SGT1 is a highly conserved single copy gene in most eukaryotes and is required for cell cycle progression at the G1/S and G2/M transitions through regulation of the kinetocore assembly and the SCF (SKP1, CDC53, F-box protein) ubiquitin ligase complex in yeast. Both AtSGT1a and AtSGT1b are able to complement temperature sensitive G1 and G2 arrest in yeast sgt1 mutants indicating that these proteins are the functional orthologs of yeast Sgt1. A series of co-immunoprecipitation experiments showed that RAR1 interacts with SGT1 in both barley and Arabidopsis. Furthermore, gene silencing of barley SGT1 and knockout studies in Arabidopsis revealed its requirement in R gene triggered disease resistance. The interaction map involving RAR1, SGT1, and SCF complex provokes models for a link between disease resistance and ubiquitination machinery in plants. This work is funded by Gatsby Foundation and BBSRC.

6-02 *TAO1* an Arabidopsis gene required for susceptible host responses to the *Pseudomonas syringae* type III effector AvrB

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Many phytopathogenic bacteria infect host plants by employing the type III secretion system to inject an array of effector proteins directly into the host cell. On susceptible host plants these effector proteins are presumed to interact with and modulate host-signaling networks for the pathogen ´s benefit. On resistant host plants these same effectors can act as elicitors of defense responses by triggering host resistance proteins. We are studying the activity of AvrB, a type III effector from Pseudomonas syringae pv. glycinea that triggers the RPM1 resistance protein on resistant Arabidopsis hosts. On susceptible hosts AvrB induces chlorosis when expressed in planta. This effect may reflect the interaction of AvrB with virulence targets in the host. To address this we initiated a conditional genetic screen to identify host mutants defective in AvrB-induced chlorosis. We identified several recessive alleles of tao1. Further characterization revealed that AvrB up-regulates and induces the proteolytic cleavage of RIN4, a component of the RPM1 resistance complex and a negative regulator of defense responses (Mackey et al, Cell 108, 2002). These effects are TAO1-dependent. In this poster we present data to suggest that TAO1 represents a link between AvrB virulence functions and regulation of components of the RPM1 recognition complex. In addition data will be presented on the cloning of TAO1 and its possible role in pathogen interactions.

6-03 *DIR1* encodes a putative lipid transfer protein involved in signaling during systemic acquired resistance in *Arabidopsis thaliana*

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Systemic acquired resistance (SAR) is abroad-spectrum plant defense response induced after a local infection by a necrotizing pathogen. SAR has been observed in many systems, but the nature and transduction of the mobile signal in the distal leaves are not yet understood. We have screened T-DNA tagged lines of *Arabidopsis thaliana* for mutants specifically compromised in SAR. The *d*efectivein *i*nduced resistance mutant *dir1-1* exhibits normal local resistance to both avirulent and virulent *Pseudomonas syringae* but fails to develop SAR to virulent strains of this bacterium or to an oomicete pathogen. Interestingly, this novel mutant exhibits wild type defense responses at the site of the initial inoculation, but the PR genes are not induced in uninoculated distant leaves. Besides, phloem exudates from wild-type SAR-induced leaves, but not from dir1-1 leaves, elicit expression of PR-1 in both wild-type and dir1-1 leaves also indicating that DIR1 has a role in the production or transmission of an essential mobile signal from the inoculated leaf to the rest of the plant. DIR1 encodes a putative lipid transfer protein. Localization of the DIR1 protein is being analyzed using a DIR1:GFP fusion and immunolocalization. We propose that the DIR1 protein may interact with a lipid-derived molecule involved in long distance signaling during SAR.

6-04 NPR1-Mediated Gene Expression in Systemic Acquired Resistance

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Systemic acquired resistance (SAR) is a secondary defense response that can be induced after a local infection. SAR is long-lasting and broad-spectrum, involving concerted activation of a large number of SAR-related genes. The Arabidopsis NPR1 protein is an essential signaling component of SAR. Mutants in the *NPR1* gene block the induction of SAR by the signal molecule salicylic acid. On the other hand, overexpression of the NPR1 protein in Arabidopsis and in rice results in enhanced disease resistance. Molecular genetic characterization showed that NPR1 is a nuclear localized protein and it regulates SAR-related gene expression through interaction with transcription factors. Using dominant-negative and chimera-reporter approaches we found that NPR1 interacts in vivo with the TGA2 transcription factor, which is a transcription activator, and regulates the binding affinity of TGA2 to DNA. To identify all the downstream genes controlled directly by NPR1, a microarray experiment has been performed. Genes regulated by NPR1 can be categorized into several functional groups and a novel promoter element has been found in the promoter region of a subset of these genes.

6-05 The *BOS1* gene encoding an R2R3 MYB transcription factor mediates biotic and abiotic stress response in Arabidopsis

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Botrytis cinerea, the necrotrophic fungal pathogen that causes the gray mould, infects a broad range of crop plants in the field and in storage, causing significant pre- and post-harvest losses. The molecular bases of host resistance to *B. cinerea* and its relationship to other defense response pathways are poorly understood. Genetic variation for response to Botrytis has been observed in Arabidopsis and other plants, but no resistance gene has been identified. We initiated forward and reverse genetic approaches to identify genes involved in resistance to *B. cinerea*. A mutant screen identified several botrytis susceptible (bos) mutants from a T-DNA mutagenized collection. We present the molecular and genetic characterization of the first of these mutants, bos1. bos1 plants have increased susceptibility to Botrytis, and to Alternaria brassicicola and Pseudomonas syringae. Interestingly, the survival of bos1 plants was significantly reduced following exposure to salt and drought stress. These and other studies established that bos1 mutant plants have altered responses to biotic and abiotic stresses. The BOS1 gene encodes an R2R3 MYB transcription factor. BOS1 expression is very low in uninfected plants and is strongly induced by Botrytis infection, with the latter being dependent on a functional COI1 allele. Since COI1 is involved in jasmonate (JA) signaling, this result suggests an interaction between BOS1 and the JA-dependent disease response pathway. In an alternate approach, we have identified candidate components of the Botrytis disease resistance signaling pathway using microarray mRNA expression profiling. These candidate components are being further tested using functional genomic approaches.

6-06 Isolation of Arabidopsis *ocp* mutants deregulated in the defense response mediated by H_2O_2

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Oxidative burst is one of the earliest responses of plants to the presence of pathogens, and therefore induced accumulation of H2O2 function as a signal for rapid deployment of defense mechanisms (including promotion of transcription) against the intruder. Here we described the specific and rapid transcriptional regulation of *Ep5c* (encoding a cationinc peroxidase) following infection with *P. syringae* both in tomato and Arabidopsis. *Ep5c* induction respond solely to H2O2 and not to other signaling molecules accumulating during the defense response. The induced expression of *Ep5c* occurs only on a local basis and irrespective of whether the plant-pathogen interaction is compatible or incompatible. We will describe also the isolation and characterization of *ocp* (overexpressor of cationic peroxidase) mutants from Arabidopsis identified in a screen for constitutive expressers of a *GUS* gene driven by the promoter of the *Ep5c* gene. These mutants show increased levels of H2O2, but only few of them show increased resistance to pathogen infection. These mutants thus offer the possibility to discriminate what part of the oxidative burst generated during defense responses contributes to the implementation of the induced resistance response.

6-07 The Arabidopsis *csb3* mutant shows increased resistance to *P. syringae* infection.

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Following pathogenic attack, plants mount defense responses with the coordinated activation of a battery of defense-related genes. The defense-related *P69C* gene, originally isolated from tomato, encodes a subtilisin-like protease. Expression of the *P69C* gene promoter fused to GUS was shown to be induced by salicylic acid as well as during the course of both compatible and incompatible interactions in Arabidopsis (1). This induced-expression takes place in both the local and the distal non-inoculated leaves but following a different and unique tissue-specific pattern of expression that is different to that described for most other defense-related genes. Here we will describe the isolation and characterization of *csb* (constitutive expression of subtilisin) mutants from Arabidopsis identified in a screen for constitutive expressers of a *GUS* gene driven by the promoter of the *P69C* gene. The recessive *csb2* and *csb3* mutants are phenotipically abnormal and show constitutive expression of a microHR program. Moreover, they show increased accumulation of SA under resting conditions along with expression of PR genes and both are resistant to *P. syringae* infection.
(1) Jorda, l. and Vera, P. (2000) Plant Physiol. 124, 1049-1058.

6-08 *Atcys* from *Arabidopsis thaliana* encodes a cysteine-protease inhibitor that functions as a negative regulator of hypersensitive cell death

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In plants, cysteine protease inhibitors (cystatins) are involved in the regulation of protein turnover and play an important role in resistance against insects and pathogens. The genome of Arabidopsis most likely contains no more than two cystatins. We have identified and characterized one member of this family (Atcys) which encodes a protein of 102 amino acids and contains the conserved motif of the cystatin superfamily (gln-val-val-ala-gly). Atcys inhibited papain activity in crude extracts from transgenic tobacco plants expressing the Atcys gene. Atcys was constitutively expressed in roots and in developing siliques. In leaves, it was strongly induced by wounding, by challenge with avirulent pathogens and by nitric oxide (NO). Overexpression of Atcys blocked cell death activated by either avirulent pathogens or by oxidative and nitrosative stress in both Arabidopsis suspension cultured cells and in transgenic tobacco plants. However, the drastic inhibition of hypersensitive cell death following infection with the avirulent pathogen did not reduce the host capacity to control the spread of pathogenic infection. Our data suggest that Atcys has a role in plant defense and argue against a general cytotoxic effect of NO in favor of its physiological function as cell death trigger through the stimulation of an active process, in which cysteine proteases appear to play a crucial role.

6-09 Priming during BABA-induced resistance in Arabidopsis

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Arabidopsis plants pre-treated with the non-protein amino-acid beta-aminobutyric acid (BABA) exhibit enhanced resistance against a variety of biotic and abiotic stresses. Recently, it was discovered that the protective effect of BABA is based on a more rapid and efficient defense response upon exposure to pathogen attack or abiotic stress. This phenomenon is known as sensitization, potentiation, or priming. To gain more understanding in the molecular mechanisms behind BABA-induced resistance, several BABA-insensitive (*bai*) mutants were isolated. Out of 15 putative mutants, three mutants, *bai24, bai38, and bai65*, were further characterized with respect to their BABA-induced priming phenotype after exposure to different biotic and abiotic stresses. Preliminary results indicate that these mutants are affected in distinct defense pathways that contribute to the priming phenomenon during BABA-induced resistance.

6-10 Disease resistance gene products Cf-9 and Cf-4 participate in membrane-associated protein complexes at one molecule per complex

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The involvement of an LRR protein in functional protein complexes has recently been described for the Arabidopsis gene Clavata2 (CLV2), and a model for its role in meristem and organ development has been presented. Interestingly, the CLV2 protein shares high structural similarity with Cf gene products and other LRR-containing receptor-like proteins (RLPs) in that it consists of predicted extracellular LRRs, flanked by cysteine pairs. Tomato Cf genes confer disease resistance to the leaf mould pathogen Cladosporium fulvum through recognition of distinct fungal-encoded avirulence (Avr) peptides secreted into the leaf apoplast during infection. CLV2 has been postulated to form an inactive disulfide-linked heterodimer of 185kDa with CLV1. Upon recognition of the ligand, CLV3, and phosphorylation of the CLV1 kinase domains, an active 450kDa complex is formed, containing the protein phosphatase KAPP and a Rho GTPase-related (Rop) protein. Given the high structural similarity shared by CLV2 and Cf- resistance proteins, together with the fact that a small extracellular peptide ligand is required in both cases to trigger the appropriate responses, it has been speculated that the Cf/Avr-dependent defense signaling is achieved via a similar mechanism. However, extensive characterization of the Cf- complex, identified by both gel filtration analysis and Blue Native gel electrophoresis, revealed essentially identical characteristics between the Cf-9 and Cf-4 complexes and significant differences from the CLV complex. Our results suggest that Cf- dependent defence signaling and CLV-dependent regulation of meristem development seem to be accomplished via distinct mechanisms despite the structural similarity of one key component.

6-11 LSD1 functions as a retention factor for the bZIP transcription factor AtCPRF2-2 in Arabidopsis

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The common plant regulatory factors (CPRFs) of parsley are transcription factors with a bZIP motif which bind to cis-regulatory elements found in promoters of genes responsive to environmental and endogenous stimuli. The results of previous studies indicated that the activity of CPRFs is triggered on different molecular levels. For instance, CPRF2 is retained in the cytosol in the dark and transported into the nucleus in response to irradiation. To analyse the mechanism of cytoplasmic retention using a broader range of applicable methods, we turned to Arabidopsis and identified three bZIP factors (AtCPRF2-1 to AtCPRF2-3) highly homologous to parsley CPRF2. Whereas AtCPRF2-1 and AtCPRF2-3 are constitutively localized in the nucleus, AtCPRF2-2 is partially found in cytoplasm. To search for protein factors withholding AtCPRF2-2 in the cytoplasmic compartment, a novel cytosolic retention screen was established in yeast. Using this assay and additional in vivo approaches, LSD1 (lesion simulating disease 1) was found to specifically retain AtCPRF2-2 in the cytoplasm of yeast and plant cells. This retention is very likely achieved by interaction of LSD1 with the C-terminus of AtCPRF2-2 thereby masking the bZIP factor´s nuclear localization signal. As LSD1 was demonstrated to be involved in the regulation of oxidative stress-dependent cell death such as occuring during the hypersensitive response (HR), we propose that at least one regulatory event in LSD1-dependent signaling consists of a pathogen-inducible nuclear import of transcription factors. Further experiments are presently in progress to unravel the molecular mechanism of LSD1-AtCPRF2-2 interaction and signaling during Arabidopsis HR.

6-12 Analysis of the IIa subgroup family of WRKY transcription factor genes in *Arabidopsis thaliana*

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The WRKY transcription factor family most likely comprises 73 functional members in Arabidopsis. WRKY factors bind to *cis* -regulatory elements designated as W boxes (C/TTGACC/T) and can function both as transcriptional activators and as repressors. Based on current knowledge, many of these plant specific factors are involved in pathogen/stress-induced signaling pathways, but have also been implicated in developmental processes. Single *WRKY* loss-of-function mutants often do not show any obvious morphological phenotypes indicating the existence of functional redundancy between certain members. Here we report on the analysis of one small WRKY subgroup gene family, designated IIa, that consist of three closely related members, *WRKY18*, *40* and *60*. Expression studies revealed similar patterns for *WRKY18* and *40* in various tissues but particularly upon treatment with salicylic acid, methyljasmonate and with bacterial flagellin, whereas that of *WRKY60* was more distinct. A *WRKY40* transposon-insertion mutant was identified but these plants showed no visible phenotypes. RNA interference was used to silence the expression of *WRKY18* and *60* in wildtype Columbia plants and in the *WRKY40* mutant. Although silencing of *WRKY60* in both cases did not lead to alterations of the phenotype, 12 out of 16 WRKY18 RNAi lines showed consistent morphological alterations but only in the *WRKY40* mutant plants. These preliminary results suggest partially overlapping functional redundancy between the WRKY18 and 40 transcription factors, a finding that we are currently trying to further substantiate. http://www.mpiz-koeln.mpg.de/~somssich/wrky_webpage/Proteomics_Homepage_Startpage.html

6-13 Characterization of ERF transcription factors induced by bacterial and fungal pathogens

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ERF proteins are novel plant transcription factors, many of which have been strongly linked to plant stress responses. We have identified a number of Arabidopsis ERF genes whose expression was specifically induced after infection by the plant pathogen Pseudomonas syringae. These genes had overlapping but distinct induction kinetics with some being induced within 3h while others were not induced till 6-12h following P. syringae infection. Infection of Arabidopsis with the fungal pathogens Alternaria brassicicola or Sclerotinia sclerotiorum also increased the RNA levels of some of these ERF genes. The expression of the ERF genes was examined among different Arabidopsis tissues, in response to the signaling molecules ethylene, methyl jasmonate (MeJA) and salicylic acid (SA) and in Arabidopsis mutants with decreased or enhanced susceptibility to pathogens and significant differences were observed. All of these ERF proteins were able to activate transcription in yeast cells. Our results suggest that transcriptional activation cascades involving ERF proteins may be important for plant defense responses to bacterial and fungal pathogens and that some ERF family members could be key players in the crosstalk between SA and MeJA signaling pathways. Reverse genetic and genomic approaches are being used to unravel the function of specific ERF genes and progress on these studies will be presented.

6-14 The Arabidopsis *obi1* mutant as a tool for the analysis of the local responses to pathogens

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Upon pathogen infection plants transcriptionally activate a number of genes that mediate in the defense response to the intruder. The expression of these pathogen-responsive genes shows both temporal and spatial hierarchy. Some undergo a rapid localized activation at the site of infection, whereas others are more slowly activated either locally and/or systemically. Many studies have concentrated on the analysis of defense-related genes in the SAR response that follows the activation of the HR program in an incompatible plant-pathogen interaction, but less is known on the defense-related genes and signal occurring during the so called Localized Acquired Resistance (LAR) response which takes place in the zone surrounding an induced HR. Here we will describe the isolation and characterization of *obi* (overexpressor of basic PR1 isoform) mutants from Arabidopsis identified in a screen for constitutive expressers of a *GUS* gene driven by the promoter of a *PR* gene from tomato encoding a basic PR-1 isoform expressed only locally at the site of pathogen inoculation. The recessive *obi1* mutant is developmentally regulated and shows no resistance differences with respect to wild-type plants when assayed using different types of plant-pathogen interactions. However, *obi1* is more prone to induce expression of SA-inducible genes and is also altered in SA and ethylene metabolism. From the phenotype of *obi1* we will try to establish parallelisms among LAR, senescence and the newly described ARR response.

6-15 Genetic analysis of avrRpm1-RPM1 interaction

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RPM1 confers resistance against isolates of the bacterial pathogen *Pseudomonas syringae* containing either the *avrRpm1* or *avrB* Type III effector genes. We have isolated mutants in Arabidopsis that no longer recognize *avrRpm1*, or fail to trigger the resistance response. These mutations fall into five complementation groups. *RPM1* is the first and more frequent target, which allowed us to make a detailed structure-function analysis. The other genes, termed Loss of Recognition to AvrRpm1 (LRA) are being analyzed. The frequency of mutations found in the different complementation groups is striking, and we will present our interpretation of this data. We cloned *LRA1* and found to be the ortholog of *RAR1* in Arabidopsis. Our progress in the characterization of the rest of *LRA* genes from a molecular, pathological and transcription profiling point of view will be discussed.

6-16 Identification and molecular isolation of *PEN2*, a gene required for nonhost disease resistance to several plant pathogens

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To identify molecular components that determine host range to powdery mildew fungi, we initiated a mutational analysis of the nonhost interaction between the grass powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*, and a model dicot plant, *Arabidopsis thaliana*. First, we characterized the plant/fungus interaction at the cytological level. Information obtained from failed infection attempts of fungal sporelings on wild type Arabidopsis enabled us to devise a simple fluorescence-based screening procedure that uses plant autofluorescence as read out to identify Arabidopsis mutants exhibiting aberrant infection phenotypes. One of the mutants, designated *pen2*, dramatically enhances the success rate of the fungus to switch from surface to invasive intracellular growth. The *pen2* mutant permits enhanced invasive growth also to other plant pathogens including the oomycete *Phytophthora infestans*. We isolated the PEN2 gene via a map-based cloning procedure. PEN2 encodes a protein with significant sequence relatedness to family 1 glycosyl hydrolases.

6-17 NPR1 modulates cross talk between SA- and JA-dependent plant defense pathways through a novel function in the cytosol

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Salicylate (SA) and jasmonate (JA) play an important role in differentially regulating induced plant defenses against pathogens and insects, respectively. Cross talk between SA- and JA-dependent pathways allows plants to fine-tune defenses, depending on the attacker encountered. Pathogen-induced systemic acquired resistance (SAR), conferring broad-spectrum resistance, is associated with the accumulation of SA and activation of genes encoding pathogenesis-related (PR) proteins. SAR-compromised NahG plants are unable to accumulate SA and do not express PR genes upon infection. Here we show that NahG plants inoculated with the pathogen *Pseudomonas syringae* pv. *tomato* DC3000 accumulated up to 25-fold more JA than wild-type Col-0 plants. Moreover, JA-responsive gene expression was enhanced as well, suggesting that in wild-type plants JA signaling is suppressed by pathogen-induced SA, thereby prioritizing the SA-dependent pathway over the one controlled by JA. Analysis of mutant *npr1*, impaired in transducing the SAR signal SA, revealed that the NPR1 protein is essential for SA-mediated suppression of JA-responsive gene expression. In the SAR reponse, NPR1 has been shown to be translocated to the nucleus where it interacts with TGA transcription factors, ultimately leading to the activation of *PR* genes. Interestingly, the promoter of the JA-responsive gene *PDF1.2* contains a TGA transcription factor binding site, potentially involved in the negative regulation of this gene. However, this binding site is not required for SA/NPR1-mediated suppression of JA-induced gene expression. Using a DEX-inducing system to control the nucleocytoplasmic localization of NPR1, we demonstrated that SA-activated NPR1 suppresses JA signaling through a novel function in the cytosol.

6-18 Towards the function of EDS5

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The Arabidopsis mutant *eds5-3* (formerly named *sid1*) was shown to be deficient in the accumulation of salicylic acid (SA) and PR1 transcript after pathogen attack and UVC-treatment. *EDS5* encodes a protein with 9 to 11 membrane-spanning domains and a coil domain at the N-terminus that is homologous to members of the MATE (multidrug and toxin extrusion) transporter family. By fusing the cDNA of *EDS5* with GFP it could be shown that the EDS5 protein is localised in the plastids of Arabidopsis. Since the biosynthetic pathway of SA in plants is not yet completely understood, the characterisation of the transport function of EDS5 might help to elucidate this important pathway. To find out if EDS5 is a transporter for a regulator or for a precursor of SA (or even for SA itself), *eds5-3* was transformed with an artificial salicylate synthase (SAS) targeted either to the plastids or to the cytosol. This enzyme consists of isochorismate synthase and isochorismate pyruvate-lyase, the last two enzymes needed for the biosynthesis of SA in *P. aeruginosa* (1). In addition EDS5-overexpressing plants were generated and analysed for their ability to accumulate SA after pathogen attack or UVC-treatment and were investigated for their resistance to pathogens.

1. Mauch F. et al. (2001). Plant J. 25:67-77.

6-19 NODO. Natural Oxylipins and Defence in Ornamentals

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Plant oxylipins are products of polyunsaturated fatty acid oxidation and comprise a wide array of molecules (e.g. fatty acid hydroperoxides, aliphatic aldehydes, oxoacids) that contribute to the key aroma and flavour compounds (6- and 9-carbon aldehydes and alcohols) and the antioxidant status of fruits and vegetables. The in vivo role of oxylipins relates to plant defence responses to pest and pathogen attack. Some of them have direct antimicrobial properties (6-C aldehydes and divinyl ethers for instance); others (jasmonic acid in particular) may act as regulators of plant defence gene expression. The low concentrations that the majority of these compounds attain in plants has so far precluded, for most of them, a direct evaluation of their involvement in plant defence responses, either as natural chemoprotectants or through regulation of gene expression. However the time is now come for using them as chemoprotectants and with constitutive expression because of developments in our understanding of the biosynthetic pathways, and the isolation and characterization of genes encoding the corresponding enzymes. The current project therefore will use both approaches. It concludes with a practical proof of concept of oxylipins as "green", environmental-friendly agrochemicals for the protection of ornamental plants against pests and diseases.

6-20 Processing in transgenic *Arabidopsis thaliana* plants of polyproteins with linker peptide variants derived from the Impatiens balsamina antimicrobial polyprotein precursor

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We have previously developed a method for expression in *Arabidopsis thaliana* of transgenes encoding cleavable chimeric polyprotein precursors. The polyprotein precursors consisted of a signal peptide and two different antimicrobial proteins (AMPs), DmAMP1 originating from Dahlia merckii seeds and RsAFP2 originating from Raphanus sativus seeds, which were linked by an intervening linker peptide sequence originating from a natural polyprotein occurring in seeds of Impatiens balsamina. By altering the amino acid sequence of the linker peptide, we now show that it is possible to improve the accuracy of polyprotein precursor cleavage, leading to the release of both AMPs with either no or a few additional amino acids derived from the linker peptide. Furthermore, subcellular localization indicated that both AMPs are predominantly present in the extracellular fluid of the transgenic plants.

6-21 Hormonal interactions in the regulation of reactive oxygen species mediated cell death

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Ozone induces HR-like programmed cell death in sensitive plants and serves as a model for cell death control by reactive oxygen species (ROS). An ozone, superoxide and avirulent pathogen hypersensitive Arabidopsis mutant, *radical-induced cell death1*(*rcd1*;Plant Cell 12: 1849) and several other signaling mutants were utilized in elucidating the interactions of ethylene (ET), jasmonate (JA), salicylate (SA), and abscisic acid (ABA) signaling with ROS in the regulation of cell death. ET enhanced cell death and functional ET signaling was required for spreading cell death while JA reduced the spreading cell death. Accordingly, ET-insensitive *ein2*, SA-insensitive *npr1* and SA-degradating *NahG* were tolerant, and ET overproducing *eto1* and JA-insensitive *jar1* and *coi1* were sensitive to ozone. 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 ozone-induced cell death. Furthermore, results indicated that JA signaling is involved in regulating plant ethylene sensitivity affecting cell death, gene expression, and also plant growth and development in general. Also, abscisic acid (ABA) is involved in ROS-mediated cell death, thus linking the processes related to osmotic stress with oxidative stress. Several ABA biosynthesis- and signaling-mutants showed a distinct cell death phenotype under oxidative stress conditions. Furthermore, the *rcd1* mutant displayed ABA-insensitivity in both gene expression and seed germination. These results suggest that RCD1 function is involved in the interaction of hormone signaling pathways.

6-22 Analysis of the signalling network in Arabidopsis mutants impaired in the accumulation of salicylic acid

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After inoculation with virulent or avirulent strains of *Pseudomonas syringae*, accumulation of salicylic acid (SA) is tightly linked to the accumulation of the pathogenesis-related protein PR-1. This was found in several Arabidopsis mutants impaired in the biosynthesis of SA, such as *pad4*, *eds5/sid1*, and *sid2*, and in salicylic acid-degrading NahG plants. However, the production of the phytoalexin camalexin is significantly diminished in NahG and *pad4* plants while normal in *sid* mutants. In order to better understand the signalling network in plant defence, four double mutants *eds5pad4*, *sid2pad4*, *eds5*NahG, and *sid2*NahG were generated. The signalling molecules SA, jasmonic acid (JA) and ethylene were measured in parallel to the production of camalexin and the expression of a number of genes involved in the pathogen response pathway in the single and double mutants. NahG as well as *pad4* were epistatic to the *sid* mutant phenotype with respect to accumulation of camalexin. The drop in accumulation of camalexin was found to correlate with a lower production of ethylene in NahG and *pad4* plants as well as in all the double mutants. The kinetics of production of JA and the expression pattern of several defence response genes is rather complex and will be presented.

6-23 Identification and characterization of Arabidopsis mutants altered in their response to a virulent strain of the bacterial pathogen, *Ralstonia solanacearum*

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The interactions between *Arabidopsis thaliana* and virulent strains of *Ralstonia solanacearum*, a soil bacterium which induces the development of the wilt disease, and found mostly in tropical and subtropical areas, are poorly understood. The *Arabidopsis-Ralstonia* pathosystem is particularly well suited for the study of disease susceptibility. In order to identify plant factors involved in wilt disease development, 25000 fast-neutron and T-DNA tagged mutants of Col-0, an ecotype susceptible to a tomato strain, GMI1000, were screened for their inability to develop wilt symptoms upon inoculation with this pathogen. Three recessive mutations have been so far identified and can be classified into 2 groups. *Err1* (enhanced resistance to *Ralstonia*) is highly resistant to all the strains of the bacteria tested so far and is not dependent on the *RRS1-R*-mediated resistance. Bacterial multiplication in this mutant is comparable to that of the resistant ecotype, Nd-1. This mutant responds in a similar way than Col-0 to inoculation by various pathogens including oomycetes, viruses, nematodes and other bacterial pathogens, suggesting that *err1* is specific of *Ralstonia*. The 2 other mutants, *err2 and err3,* are resistant to all the pathogen strains which are avirulent on Nd-1 and virulent on Col-0. However, these 2 mutants remain susceptible to some *Ralstonia* strains which are virulent on Nd-1. Our results suggest that these 2 mutations affect the signalling pathways leading to *RRS1-R*-mediated resistance. The response of these 2 mutants to other pathogens is currently being tested and their characterization will be presented.

6-24 Analysis of the interaction between plants expressing a fungal cutinase and several fungal or fungal-like pathogens

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The cuticle is the interface between the plant and its environment. Transgenic plants expressing the cutinase of *Fusarium solani* f. sp. *pisi* have been generated to study the functions of cutin, the major structural polymer of the cuticle. Such plants have a modified ultrastructure and an increased permeability of the cuticle, and strong post-genital organ fusions. Thus, the cutin layer plays an important role as a permeability barrier and in the development of the plant. The cuticle is the first physical barrier that phytopathogenic microorganisms have to overcome. How do cutinase-expressing plants react when they are infected with different pathogens? No difference was observed between cutinase-expressing plants and wild-type plants when infected with the non-host pathogen *Blumeria graminis* and both were found to be similarly susceptible to the obligate biotrophs *Erysiphe cichoracearum* UCSC1 and *Peronospora parasitica* NOCO, and to the non-obligate biotroph *Phytophthora porri* strain D. However, they show a strong resistance to the necrotrophic fungus *Botrytis cinerea* strain BMM. Cutin monomers have been reported to induce defense reactions in plants. The resistance of cutinase-expressing plants to. *cinerea* is neither correlated to a mecanism of potentiation of the accumulation of *PR-1* and *PDF1.2* mRNAs, nor to the constitutive expression of these defense genes. The germination of spores is not inhibited on cutinase-expressing plants, but the growth of the hyphae is slower and stops after a few days.

6-25 Characterisation of a new type of ß-thioglucoside glycosidase (myrosinase) in *Arabidopsis thaliana*

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Myrosinases are ß-thioglucoside glucosidases (EC 3.2.3.1) that catalyse hydrolysis of secondary metabolites called glucosinolates (anionic 1-thio-ß-D-glucoside N-hydroxysulphates). This binary system is present in Capparales and serve as an important defense barrier to many pests. Until now three myrosinase genes has been reported in *Arabidopsis thaliana*. As an accomplishment of the genome sequence project, three new potential genes were found to be similar to the earlier described myrosinase genes, *TGG1, TGG2 and TGG3*. These novel genes were denoted as *TGG4, TGG5 and TGG6*. The sequence identities between the new group and the known myrosinase genes are below 60%. These novel myrosinases were not detected by Southern blotting using probes designed to the earlier characterised myrosinase genes. We cloned the full-length coding cDNA sequence of TGG4, and after overexpression of the TGG4 cDNA in Pichia pastoris myrosinase activity was recorded for the recombinant enzyme. A monoclonal antibody recognizing TGG1 and TGG2 did not react with the TGG4 protein. RT-PCR based tissue expression analysis of TGG4 transcripts revealed them be root specific. The TGG4 gene was found to differ from the known myrosinase genes in several aspects. It contains 13 exons while all other known myrosinases share a consensus number of 12 exons, they have longer and diverged signal peptides, and the unusual GC intron splice sites are used in different positions. All these features suggest that TGG4, TGG5 and TGG6 belong to a new subfamily of myrosinase genes, which also is strongly supported by phylogenetic analysis.

6-26 EDS5, an essential component for induced salicylic acid accumulation in Arabidopsis

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EDS5, an essential component for induced salicylic acid accumulation in Arabidopsis Christiane Nawrath, Sarah Wegmüller, Silvia Heck, Nonglak Parinthawong and Jean-Pierre Métraux Two mutants that do not accumulate salicylic acid (SA) after exposure to biotic or abiotic stresses, named *sid1 and sid2* (for SA induction-deficient) display a loss in PR-1 accumulation after pathogen inoculation and an increased susceptibility to bacterial and fungal-like pathogens (Nawrath and Métraux, 1999). *sid1* was found to be allelic to *eds5* (Rogers and Ausubel, 1997). *EDS5* has been cloned by a map-based cloning procedure and encodes for a protein with predicted 9-11 membrane spanning domains belonging to the family of MATE (for multi drug and toxin extrusion) proteins that transport organic molecules (Nawrath et al., 2002). EDS5::GFP fusions indicate that EDS5 is a transporter located at the plastid. This is interesting, since SID2 is predicted to be a plastid-located isochorismate synthase directly involved in induced SA biosynthesis from the plastid-located precursors of the skikimate pathway (Wildermuth et al., 2001). Which molecule EDS5/SID1 transports and why the transport deficiency leads to the *sid* mutant phenotype is currently under investigation. Analysis of the transcriptional regulation of *EDS5* revealed that *EDS5* is rapidly induced by SA-inducing treatments, such as pathogen inoculation and exposure to UV-C light, before accumulation of SA is observed. In addition, expression of *EDS5* is strongly dependent upon three proteins previously found to be involved in pathogen response EDS1, PAD4 and NDR1, either after pathogen inoculation or treatment with UV-C light.

6-27 Constitutive expression of *ETHYLENE-RESPONSE-FACTOR1 (ERF1)* in arabidopsis confers resistance to several pathogens

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ERF1 is an early transcription factor gene whose expression is regulated by EIN3 and, in turn, regulates the expression of several pathogen responsive genes, including b-CHI and PDF1.2. We have previously described the role of ERF1 as a regulator of ethylene responses after infection of Arabidopsis with the necrotrophic fungi Botrytis cinerea and Plecthosphaerella cucumerina (Berrocal-Lobo et al., 2002). ERF1 expression is induced after infection with these fungi, and transgenic overexpression of ERF1 in Arabidopsis is sufficient to confer resistance to these fungi (Berrocal-Lobo et al., 2002). Now we have extended our analysis of Arabidopsis disease resistance to the fungus Fusarium oxysporum and the bacteria Erwinia carotovora. A positive co-operation between ethylene, salicylic acid and jasmonate signal transduction pathways was observed in plant response to F. oxysporum. In plants infected with this fungus or E.carotovora the expression of ERF1 transcriptional factor is upregulated. Furthemore, transgenic plants overexpressing ERF1 show enhanced resistance to these pathogens. These results indicate that ERF1 is a key regulator of activation of Arabidopsis defence mechanism against these pathogens.

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6-28 RPP13-ND: An Arabidopsis *R* gene product that acts via a novel signalling pathway

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Previously we have demonstrated that the *RPP13-Nd*-mediated resistance to *Peronospora parasitica*, an obligate biotrophic oomycete, is not compromised in a background of salicylic acid depletion (*nahG*) and it functions independently of *NDR1* and *EDS1*, (Bittner-Eddy and Beynon, 2001). Furthermore, *pbs2, pad4-1, npr1-1 and rps5-1*, which compromise resistance to a number of *P. parasitica* isolates, had no effect on *RPP13-Nd* function. These observations suggest that RPP13 acts via a novel signalling pathway. Currently we are screening EMS-mutagenised seed from a Col::*RPP13-Nd* transgenic line to identify components of such a pathway. Also, a recombinant inbred line (HRI3895), that has been developed from a cross between Arabidopsis accessions Col-5 and Nd-1 (Bittner-Eddy et al., 1999), has been shown to support a limited amount of sporulation (1-10 sporangiophores). This phenotype is inconsistent with either parental accession following inoculation with Maks9, Edco1, Emco5, or Goco1, although HRI3895 contains the wild type form of *RPP13*. This result implied that another locus, essential for the function of *RPP13*, is missing (either due to a recombination or mutation event) from HRI3895. In crosses this susceptibility segregates as a single recessive locus and, therefore, probably represents a component of the *RPP13* signal transduction pathway. We are currently trying to identify this second component.

6-29 A novel lipase-encoding pathogen-inducible gene family of Arabidopsis

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In a differential screening between Arabidopsis plants pretreated with the resistance-inducer BABA and untreated control plants, we have isolated a gene, *PRLIP1*, encoding a novel lipase-like protein. *PRLIP1* belongs to a gene family comprising 6 (*PRLIP1, PRLIP2, PRLIP4, PRLIP5, PRLIP6, PRLIP7*) closely related genes in tandem position on the chromosome 5, and they are unique for Arabidopsis. *PRLIP3* and *PRLIP8* are more distantly related members of this family on the chromosome 2 and 5, respectively. Orthologues of these two latter genes were found in rice. The *PRLIP* genes show tissue-specific expression pattern. The abundance of *PRLIP1* mRNA is up-regulated by application of BABA, SA and ethylene as well as by various abiotic and biotic treatments, such as high humidity, infection with *Peronospora parasitica, Pseudomonas syringae pv tomato (Pst), Plectosporium cucumarium, Phytophthora porri. PRLIP2* is induced by SA, BTH and pathogens, but on a much lower level than *PRLIP1. Botrytis cinerea*, however, does not induce these genes. *Pst* avrRpt2 (incompatible interaction) induced expression of *PRLIP1* in different signalling mutant plants (nahG, *npr1, cpr1, etr1, jar1*) is altered demonstrating the SA and ethylene dependent expression of this gene. The PRLIP1 protein produced by *E.coli* showed lipase (esterase) activity on lipolic substrates such as Tween20, Tween80 or p-nitrophenil-butyrate. The potential role of the *PRLIP* genes in disease resistance will be discussed.

6-30 A genetic screen for suppressors of *sni1*

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Systemic acquired resistance (SAR) is a general defense mechanism that confers long lasting and broad-spectrum plant disease resistance. SAR requires salicylic acid (SA), and is associated with the induction of pathogenesis related (*PR*) genes, the concerted action of which is thought to result in resistance. The *NPR1* (non-expresser of *PR* genes) gene was identified in *Arabidopsis thaliana* as a positive regulator of SAR. *npr1* mutants fail to express *PR* genes and show increased susceptibility to pathogens. In contrast, *NPR1* over-expression results in stronger *PR* gene induction after infection and confers disease resistance with no detrimental effects to the plant. A suppressor screen carried out in the *npr1-1* background identified the recessive *sni1* mutant, which restores SA-inducible *PR* gene expression and pathogen resistance. This phenotype suggests that the wild type SNI1 protein is a negative regulator of SAR. To increase our understanding of SNI1 function, and identify other components of the SAR signaling network, a screen for mutants that suppress the *sni1* mutation was carried out. The *sni1* mutant is much smaller than wild type plants and expresses *PR* genes at a low level in the absence of SAR induction. We took advantage of these phenotypes by looking for mutants that are similar in size to wild type plants, in a homozygous *sni1* background. Candidates were then tested for *PR* gene expression. Twenty candidates have been identified that suppress both the morphology and background *PR* gene expression of *sni1*. The phenotypes of these *sni1* suppressors will be discussed.

6-31 Identification of mutants impaired in NIM1/NPR1-independent pathogen-induced transcription

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Salicylic acid (SA) and the NIM1/NPR1 protein are important regulators of defense responses in Arabidopsis– both molecules have been shown to be necessary for the accumulation of many PR gene transcripts, the resulting expression of systemic acquired resistance, and in some cases, race-specific resistance. Plants lacking SA or NIM1/NPR1 function are not completely impaired in defense responses, suggesting that there are pathogen-induced pathways operating independently of these two molecules. We have found that most race-specific resistance to *P. syringae*, and *P. parasitica* requires SA, but not NIM1/NPR1, and we have identified a number of NIR (NIM1/NPR1-Independent Response) genes that require SA but not NIM1/NPR1 for their pathogen-elicited expression. In an attempt to determine if NIR genes play a role in race-specific resistance or other defense responses, we have screened for mutants unable to express a GUS reporter construct under regulation of the *NIR1* promoter. We have identified several *nue* (NIR1-underexpressor) mutants; one of these, *nue2*, is compromised in RPP4-mediated resistance to *P. parasitica* isolate Emwa, suggesting a role for NIM1/NPR1-independent transcriptional regulation in race-specific resistance.

6-32 SNI1, A Negative Regulator of Systemic Acquired Resistance

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Systemic Acquired Resistance (SAR) is characterized by an increase in salicylic acid (SA) levels which causes upregulation of pathogenesis-related (*PR*) genes and resistance to a broad range of pathogens. A critical positive regulator of SAR, *NPR1* (non-expressor of *PR* genes), is known to act downstream of the SA signal and upstream of *PR* gene induction. *npr1* mutants exhibit neither *PR* gene expression nor systemic resistance in response to SA treatment. In the *npr1* mutant background, *sni1* (suppressor of *npr1-1*, inducible) restores inducible *PR* gene expression and resistance. SNI1 is hypothesized to be a transcriptional repressor present at the promoters of *PR* genes, which is removed by NPR1 upon induction of SAR. The SNI1 sequence has no obvious homology or known domains, though putative orthologs have been found in other plant species. Functional analysis of the SNI1 protein, including scanning mutagenesis, will be discussed.

6-33 Non-host resistance in the Arabidopsis powdery mildew disease monitored by microarrays

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Plants are constantly exposed to a wide variety of pathogens. When a plant species is sensitive to a pathogen, this species is termed a host for this pathogen. However, few pathogens successfully invade a given plant species and resistance is generally observed. This broad spectrum resistance is called non-host resistance. Arabidopsis is a host to the powdery mildew *Erysiphe cichoracearum* and a non-host to *Blumeria graminis* f.sp. *hordei* (*Bgh*). *Erysiphe cichoracearum* successfully infects Arabidopsis by developing haustorium followed by hyphal growth and conidiation. In contrast, the barley powdery mildew (*Bgh*) does not infect Arabidopsis. Although *Bgh* spores successfully attach, germinate and develop appressoria on Arabidopsis leaves, the great majority of the spores fail to penetrate. We took advantage of Arabidopsis microarrays generated as part of the Arabidopsis Functional Genomics Consortium (AFGC, http://afgc.stanford.edu/) to compare the global gene expression patterns during host and non-host interaction. We used two types of microarray consisted of either a set of 11,300 ESTs or 14,300 clones. Poly(A)+ RNA was extracted at 8, 18 and 24 hours post inoculation from Arabidopsis leaves inoculated with either *E. cichoracearum* or *Bgh*. These time points correspond to mature appressoria for both pathogens, first haustorium fully develop during *E. cichoracearum* infection and callose deposition around attempting penetration by *Bgh*, respectively. Analysis of the arrays data identified more than 50 genes with > 2 fold response to both pathogens. Detailed analysis of the results of these experiments will be presented.

6-34 How does *Pseudomonas syringae* avrPphB trigger RPS5-mediated resistance?

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RPS5, *PBS1* and *PBS2* (*AtRAR1*) are essential for Arabidopsis resistance to the bacterial pathogen *Pseudomonas syringae* carrying the avirulence factor avrPphB (1). RPS5 belongs to the NBS-LRR class of disease resistance proteins. We hypothesize that the protein kinase PBS1 is part of an avrPphB recognition complex in which a specific modification of PBS1 by avrPphB triggers RPS5 function. Consistent with this model, PBS1 is specifically required for the avrPphB/RPS5-mediated resistance; PBS1 and RPS5 co-localize in the microsomal fraction of naïve plants, and the myristoylation site of avrPphB is required for its plasma membrane localization and RPS5 recognition. Co-immunoprecipitation experiments are in progress using Agrobacterium-based transient expression assays. Preliminary data suggest that PBS1 is degraded after avrPphB elicitation. In collaboration with Dr. Jack Dixon?s laboratory, we have shown that avrPphB is a cysteine protease whose activity is required for self-processing and plant hypersensitive response induction (2). We are currently investigating whether avrPphB processes PBS1, leading to its degradation, and whether this degradation requires AtRAR1. We are also using a yeast-two hybrid system to look for Arabidopsis proteins that interact with a protease-inactive form of avrPphB. Finally, microarray analysis suggests PBS1 is a negative regulator of basal defense. A model integrating these data will be presented.

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6-35 A search for mutations that disrupt NIM1-independent disease resistance

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Multiple pathways contribute to disease resistance in Arabidopsis. The *NIM1/NPR1* gene has been found to be a key regulator of systemic acquired resistance (SAR), which is activated in plants after inoculation with pathogens or exposure to chemical inducers of SAR, such as salicylic acid and INA. The *NIM1/NPR1* gene has also been implicated in another form of resistance called induced systemic resistance (ISR), expressed in plants whose roots were colonized by the non-pathogenic rhizobacterium *Pseudomonas fluorescens* strain WCS417r. The *nim1/npr1* mutants are also more susceptible to compatible and some incompatible pathogens, showing a role for the *NIM1/NPR1* gene in race-specific resistance, and recent studies have shown that different resistance genes have different requirements for a functional *NIM1/NPR1* gene. We hypothesize that in the absence of a functional *NIM1/NPR1* gene, other signal transduction pathways involved in disease resistance will be exposed and thus accessible to genetic analysis. Therefore, to identify mutations that disrupt NIM1/NPR1-independent resistance pathways, we conducted genetic screens in a *nim1-1* (Ws-0) background, and sought for mutants that displayed enhanced susceptibility to the normally incompatible oomycete parasite *Peronospora parasitica* isolate Noco2. Four mutants have been found in this screen and their initial characterization will be presented.

6-36 Analysis of NIM1/NPR1 protein phosphorylation and function in systemic acquired resistance

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The Arabidopsis NIM1/NPR1 protein is essential for the induction of systemic acquired resistance (SAR) by pathogen elicitors, salicylic acid (SA) or synthetic analogues of SA. Plants expressing SAR display broad-spectrum disease resistance and show increased levels of pathogenesis-related gene expression, such as PR-1. NIM1/NPR1 shows sequence simility to other ankyrin repeat containing proteins, such as Ik-Ba, a class of regulatory proteins that in animals control the innate immune system. Although there is some information about NIM1/NPR1's mode of function, such as its localization and interactions with some transcription factors, little is known about how NIM1/NPR1 regulates responses to signals that activate PR gene expression or SAR. Based on the regulation by phosphorylation of Ik-B and related proteins, such as Cactus in *Drosophila*, we examined phosphorylation of NIM1/NPR1 using plant extracts combined with NIM1/NPR1 made in *E. coli*. We conducted phosphoamino acid analysis to identify which amino acids were modified and phospho-peptide mapping to locate the sites where the protein was modified. To test whether NIM1/NPR1 phosphorylation is important for SAR, forty site directed mutations were created that replace potential phosphorylation sites with alanine. The NIM1/NPR1 mutant genes were tested in an Arabidopsis protoplast transient assay with a PR-1 promoter-GUS reporter system. By correlating which amino acids are phosphorylated, with transient expression data that tests functionality of the alanine substitution mutant proteins, we hope to determine whether phosphorylation plays a regulatory role in NIM1/NPR1 function. The most interesting mutations are in testing in transgenic nim1-1 plants.

6-37 *EDS1*, *PAD4* and *NDR1* are required for pathogen resistance but not for SA accumulation, spontaneous lesion formation and *PR-1* gene expression in the Arabidopsis mutant *cpr22*

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To investigate the signaling pathways through which defense responses are activated following pathogen infection, we have isolated and characterized the *cpr22* mutant. The *cpr22* mutant expresses pathogenesis-related genes constitutively and shows elevated levels of salicylic acid (SA), spontaneous lesion formation and heightened resistance to an oomycete pathogen, *Peronospora parasitica*, and a bacterial pathogen, *Pseudomonas syringae*. Further characterization of enhanced resistance conferred by *cpr22* was conducted by carrying out epistatic analysis with Arabidopsis mutants defective in *R* gene-mediated signaling. We constructed three double mutants: *cpr22 eds1*, *cpr22 pad4*, and *cpr22 ndr1* and analyzed them for pathogen resistance. It has been reported that *EDS1*, *PAD4* and *NDR1* play important roles in limiting pathogen growth in *R* gene-mediated resistance. The requirement for *EDS1* (*PAD4*) or *NDR1* is largely determined by the structure of the *R* gene, arguing that at least two different signaling pathways are involved in *R* gene-mediated resistance. Interestingly, all three double mutants lost the heightened resistance to oomycete or bacterial pathogens exhibited by *cpr22*. However, they had all other *cpr22*-related phenotypes including elevated levels of SA. Nonetheless, SA was found to be necessary for *cpr22*-conferred resistance, using *cpr22* plants carrying the *nahG* transgene, which suppresses SA accumulation. Taken together, these results argue that SA accumulation is required, but is not sufficient, for enhanced pathogen resistance conferred by *cpr22*; a second signal that is affected by *eds1*, pad4 and ndr1 is also required. The CPR22 gene has been cloned and functional analysis of this gene is currently in progress.

6-38 Characterization of *Arabidopsis thaliana* plants that express AvrPto of *Pseudomonas syringae* pv. Tomato strain DC3000

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Although much research has focused on disease resistance mechanisms, the molecular basis of plant susceptibility to pathogen infection remains elusive. We are investigating the susceptibility of Arabidopsis to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pst* DC3000). *Pst* DC3000 causes speck disease, characterized by water-soaking, chlorosis, and necrosis in the infected leaves. *Pst* DC3000 is believed to deliver numerous effector proteins, via the type III secretion system, into host cells to promote disease. Some of these "type III effector proteins" act as avirulence factors in resistant plants. One of the most extensively characterized avirulence factors is AvrPto, which activates Pto-dependent resistance responses in tomato. Whether AvrPto has a role in virulence in susceptible Arabidopsis has not been determined. One method of determining the role of AvrPto in a susceptible host is to create and analyze transgenic plants that express this bacterial protein. Several Arabidopsis lines expressing AvrPto under the dexamethasone-inducible promoter have been generated. Microarray analysis showed that the gene expression pattern of plants expressing AvrPto was similar to that of plants infected by *Pst* DC3000. In addition, growth of several non-pathogens was greater in AvrPto-expressing plants compared to that in wild-type plants. These results suggest that overexpression of AvrPto is sufficient to enhance Arabidopsis susceptibility to non-pathogens.

6-39 Characterization of three Type III effectors of *Pseudomonas syringae* pv. tomato DC3000

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Pseudomonas syringae pv. *tomato* DC3000 (*Pst* DC3000) is a virulent pathogen of *Arabidopsis thaliana* and uses the Type III system to deliver effector proteins into host cells. These effector proteins, more commonly known as Avr proteins, were initially identified as mediators of gene-for-gene resistance responses. Increasing evidence supports dual functions for *avr* proteins as recognition determinants in resistant hosts and virulence determinants in susceptible hosts. These effectors are believed to collectively play a key role in bacterial virulence in a host. Three new effector genes were identified by screening a cosmid library of *Pst* DC3000 using all known *P.syringae* avr genes as probes. They have been named virPtoA, *avrPtoE*, and *avrPtoB* and are homologues of *virPphA* and *avrPphE* from *Pseudomonas syringae* pv. *phaseolicola*, and *avrPpiB* from *Pseudomonas syringae* pv. *pisi* respectively. All three genes encode hydrophilic proteins. Of the three, *virPtoA* and *avrPtoE* have the conserved harp box motif in their promoters. We have generated insertion mutants of all three effectors to investigate a possible role in virulence. A yeast two-hybrid screen is currently in progress to identify interacting host proteins. We are also using a *Pseudomonas fluorescens* - based Type III delivery system to study the effect of individual effector proteins on host gene expression virulence. Characterization of these mutants and expression profiling analysis of the three effectors will be presented.

6-40 Isolation of a novel protein kinase interacting with cucumber mosaic virus 1a

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The cucumber mosaic virus (CMV)-encoded 1a protein has been implicated to play a role in viral replication. However, it is unknown whether any intrinsic components of plant cell participate in this process. In this study, we isolated a putative Arabidopsis protein kinase, AtCOIK1 that interacts with CMV 1a by the yeast two-hybrid system. Nucleotide sequencing and database searches revealed that AtCOIK1 had a typical protein kinase domain. The RT-PCR results showed that transcripts corresponding to AtCOIK1 were expressed constitutively in response to CMV infection. Especially, AtCOIK1 showed higher expression in Col-0 ecotype than in C24 ecotype, resistance type to CMV infection. AtCOIK1 interacted with N-terminal region of CMV 1a, but not C-terminal region. This result suggested that the methyltransferase domain of CMV 1a participated in interacting with AtCOIK1. AtCOIK1::DsRFP was distributed over the endoplasmic reticulum (ER), with strongest accumulation on the perinuclear region of the ER. This implicated that AtCOIK1 and CMV 1a colocalize on ER and AtCOIK1 is capable of interacting with CMV 1a.

6-41 Brassinosteroids induce disease resistance in Arabidopsis

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Brassinosteroids (BRs), a class of phytohormone, induce a broad range of disease resistance in tobacco and rice, as reported previously. To clarify the induction mechanism of the resistance mediated by BRs, the effect of brassinolide (BL), the most active BR, on Arabidopsis plants was investigated. Arabidopsis ecotype Columbia was treated with BL and inoculated with a virulent bacterium *Pseudomonas syringae* pv. *tomato* DC3000. The result showed that the treatment of BL significantly reduced the bacterial growth in plant tissues. BL could induce disease resistance in NahG transgenic plants. BL treatment did not induce the accumulation of salicylic acid in wilt-type plants. Thus BL-induced resistance dose not require SA biosynthesis. RNA analysis revealed that the treatment of BL did not induce the expression of *PR-1* gene, indicating that BL-induced resistance is distinct from systemic acquired resistance (SAR). Experiments using SAR inducers indicated that BL-induced resistance and SAR have the additive protective effect against pathogen. It is reported that the BL induces the expression of jasmonic acid (JA) biosynthetic genes, however, BL induced the resistance in a JA-insensitive Arabidopsis mutant *jar1*. This indicated that BL-induced resistance is different from induced systemic resistance (ISR) meditated by non-pathogenic rhizobacteria. These data suggests that BL-induced resistance is a novel type of resistance distinct from SAR and ISR.

6-42 Activation-tagged mutants restore resistance to the *rps2* mutant and the *nahG* transgenic line

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In Arabidopsis, *R*-gene mediated defense against *Pseudomonas* bacteria is dependent on R-proteins, like RPS2 and salicylic acid. Accordingly, *rps2* mutant plants or the *nahG* transgenic line display a strong susceptible phenotype after exposure to *Pseudomonas syringae* pv. *tomato* expressing the avirulence gene *avrRpt2* while wild-type Col-0 plants remain asymptomatic green. We used activation-tagging to identify mutants, which suppress the susceptible phenotype. Screening some 80,000 Basta resistant *nahG* and 50,000 Basta resistant *rps2* plants, we so far isolated 8 mutants in the *nahG* background and 6 in the *rps2* background which do not show the leaf-yellowing symptoms of the parental lines. With the *asn* mutants (activation suppressors of *nahG*) we hope to reveal components of salicylic acid-independent defenses, while the *asr* mutants (activation suppressors of *rps2*) might identify components close to the *R*-gene *RPS2*, which when over-expressed activate defense even in the absence of *R*-gene derived signals. Presently, we are isolating homozygous lines for each of the mutants to perform on these the molecular and physiological characterization. Progress on these studies will be reported.

6-43 Expression profiling of the complete set of glutathione transferases, glutathione peroxidases and glyoxalases in *Arabidopsis thaliana*

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A DNA array was constructed to study the expression of genes encoding glutathione (GSH)-utilizing enzymes. The array contained gene specific probes for 44 GSH-transferases (GSTs), 8 GSH-peroxidases and 6 glyoxalases. Expression profiling of Arabidopsis inoculated with *Pseudomonas syringae* pv *tomato* DC3000 (±*avrRpt2*) revealed that the expression of 12 GSTs, 3 GSH-peroxidases and 1 glyoxalase were up-regulated in response to pathogen attack. Interestingly, the pathogen-induced GSTs belonged to the three different GST subfamilies phi, tau and zeta. To learn more about the regulation of these genes expression profiling with ethylene-, salicylic acid- and methyl jasmonate-treated Arabidopsis was performed. With two exceptions, all of the pathogen-induced GST-genes were either induced by ethylene, SA and/or methyl jasmonate. However, the expression of 5 pathogen-induced genes including 1 glyoxalase, 2 GSH-peroxidases and 2 GSTs, was not up-regulated by either hormone. This result suggests that additional not yet known signaling mechanisms mediate the expression of this group of genes in response to P. syringae.

6-44 ISR mediated by *Pseudomonas fluorescens* CHA0: A different aspect of ISR

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Plant-growth-promoting rhizobacteria (PGPRs) are used as inoculants for biofertilization, phytostimulation and biocontrol. Induced systemic resistance (ISR) represents one of the mechanisms responsible for the biocontrol activity of PGPRs against a broad spectrum of pathogens. Various Pseudomonas rhizobacteria have the ability to induce ISR in different plant species, under conditions in which the bacteria remain spatially separated from the challenging pathogen. In spite of the similarity of ISR with pathogen-induced systemic acquired resistance (SAR) it seems that ISR acts through a different signalling pathway than SAR. ISR was studied in Arabidopsis using *Pseudomonas fluorescens* strain CHA0 as biocontrol agent. This offers the advantage that various described mutants are available both on the plant and the biocontrol agent side. The effectiveness of the CHA0 strain was mainly tested against the oomycete *Peronospora parasitica*. This system leads to a 20-30% reduction in infection, whereas among all the Arabidopsis mutant tested only *jar1-1* failed to restrict the fungal growth. Moreover we observed that two mutants of CHA0, both described as deficient in 2,4-diacetylphloroglucinol (DAPG), exhibit incapacity to express ISR. Currently, we are investigating the possible role of DAPG as an ISR-eliciting compound and jasmonate as molecule engaged in the ISR signalling pathway. Our findings are only partially similar to those already described in the literature, confirming the impression that ISR might be more complex than hitherto described.

6-45 Towards new components of flagellin signalling

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Effective defense mechanisms of plants depend on early recognition systems for characteristic structures of invading pathogens. Detection of the elicitor flg22, a peptide derived from the most conserved domain of bacterial flagellin, cause rapid changes in protein phosphorylation, and typical defense responses such as oxidative burst and *PR* gene expression, paired with an inhibition of seedling growth. In Arabidopsis, the receptor-like kinase FLS2 is the major component of the flg22 perception process. Plants harboring mutations in the *FLS2* gene show both, flg22 insensitivity in terms of oxidative burst and growth inhibition, and loss of flg22-binding capability. Further transmission of the flg22 signal requires a MAP kinase cascade, and likely involves gene regulation mediated by WRKY transcription factors. We are addressing the link between flg22-activated MAP kinase cascade and flg22-induced transcriptional changes. 1) A proteomic approach is used to screen for nuclear proteins, which act as downstream acceptors of flg22 signalling and function as key regulators in mediating of immediate-early flg22-induced gene expression. 2) Mutant plants impaired in defense response pathways and hormone signalling pathways are tested for their flg22 response in order to identify known regulatory components that may also affect flg22 signal transduction.

6-46 Natural variability in Arabidopsis-necrotrophic fungi interactions

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Necrotrophic fungal pathogens are responsible for major losses in yield and quality in some crops, and potentially can cause severe epidemics. Plant resistance against necrotrophic fungi is considered to be unspecific and their genetics remains almost unknow. In an attempt to get inside the genetic basis of necrotrophic fungi-plant interaction, we have taken advantage of the natural variability of Arabidopsis by screening different accesions for resistance/susceptibility to the necrotrophic fungi *Plectosphaerella cucumerina*, *Fusarium oxysporum* and *Botrytis cinerea*. Resistant and susceptible accesions to these pathogens have been identified. A QTL analyses using recombinant inbred lines has been initiated to identify genes involved in Arabidopsis resistance against these fungi. The expression patterns of some well-known defence genes as *PR1*, *PDF1.2* and *TH2.1* has been analysed in these resistant and susceptible accesions after infecton with *P. cucumerina*; so far, a clear correlation between resistance and the expression level of these defense genes has not been found.

6-47 Ethylene and jasmonic acid signaling in response to pathogens in Arabidopsis. Role of ERF1

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Ethylene (E) and jasmonic acid (JA) have been shown to participate in pathogen responses in Arabidopsis. To further clarify the role of these hormones in pathogen defense we have investigated the implication of *ETHYLENE-RESPONSE-FACTOR1 (ERF1)* in these responses. Gain-of-function experiments demonstrated that *ERF1* is sufficient to confer resistance to several necrotrophic fungi (*Botrytis cinerea* and *Plectosphaerella cucumerina*). *In vivo*, ERF1 is rapidly induced upon infection with *B. cinerea* and regulates the expression of pathogen response genes. Gene-expression profiling of ERF1-overexpressing transgenic plants, showed that over 60% of the genes induced by E and JA simultaneously, are constitutively expressed in ERF1-expressing transgenic plants explaining, at the molecular level, the cause of the resistance. A positive co-operation between E and SA pathways was observed in the plant response to *P. cucumerina*. Infection by *Pseudomonas syringae tomato* DC3000, however, does not affect *ERF1* expression, and activation of ethylene responses by *ERF1* -overexpression in Arabidopsis plants reduces tolerance against this pathogen, suggesting a negative crosstalk between E and SA signaling pathways, and demonstrating that positive and negative interactions between both pathways can be stablished depending on the type of pathogen. To further understand the role of JA in pathogen resistance we have set up a new screening and found three new JA-insensitive mutants, namely *jai1*, *2* and *3* (*jasmonic acid insensitive*). The cloning and molecular characterization of the corresponding genes is currently underway.

6-48 Identification of Arabidopsis mutants with spontaneous lesion formation

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To better understand the mechanisms controlling the activation of a hypersensitive reaction (HR), we have screened a population of M2 mutagenized plants containing a selectable marker fused to plant regulatory sequences responding to salicylic acid (SA), nitric oxide (NO) and reactive oxigen species (ROS), three signal molecules required to promote HR cell death. Two mutants dge4 and dge9 (dge, for defence gene expressers) have been characterized. The dge4 mutant displayed spontaneous formation of hypersensitive lesions, as well as activation of molecular markers associated with oxidative stress and production of SA. Differentially, the dge9 mutant develops a chlorotic phenotype and is defective in the activation of specific SA-responsive genes. Correlated with these results, the dge4 plants showed an increased resistance to Pseudomonas syringae infection and enhanced susceptibility to fungal pathogens such as Alternaria brassicicola and Botrytis cinerea. On the other hand, the dge9 mutant exhibits more susceptibility to both bacterial and fungal pathogens. The two mutations have been localized in the Arabidopsis genome. The dge4 locus mapped to chromosome II and the dge9 mutant to chromosome IV.

6-49 Fatty acid alpha-dioxygenases: Their role in plant defense

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alpha-dioxygenase (alpha-DOX1) is a recently discovered enzyme which catalyzes the primary oxygenation of fatty acids acids into their 2(R)-hydroperoxy derivates. This compound is spontaneously converted by into classical alpha-oxidation products, i.e., a mixture of the corresponding lower aldehyde (Cn-1), the lower fatty acid (Cn-1) and a Cn 2-hydroxy fatty acid. (Sanz et al., The Plant Cell, 10: 1523-1537; Hamberg et al., J. Biol. Chem. 274: 24503-24513). The Arabidopsis alpha-DOX1 gene is induced in response to inoculation with different Pseudomonas strains. In addition, gene expression is also activated in plants responding to infection with necrotrophic pathogens such as Erwinia carotovora subsp. carotovora and Botrytis cinerea. Accumulation of gene expression is impared in SA-compromised plants and induced by SA, and by chemicals generating NO and intracellular superoxide. The role of alpha-dioxygenase in plant defense has been examined. The antimicrobial activity of alpha-dioxygenated-derived oxylipins has been examined in vitro. Furthermore, transgenic Arabidopsis plants possesing elevated or reduced levels of alpha-dioxygenase activity have been characterized. Data from these analyses suggest that plant alpha-dioxygenases are used to generate lipid-derived molecules for a process that protects plant tissues from oxidative stress and cell death (Ponce de León et al., The Plant Journal 29(1): 61-72, 2002).

6-50 Overexpression of RPW8 Leads to Conditional Spontaneous Cell Death and Altered Response to Pathogens

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The novel class of *R* gene *RPW8* from Arabidiopsis confers broad-spectrum powdery mildew resistance via salicylic acid (SA)- and *EDS1*-dependent defense responses including hypersensitive response (HR), similar to that controlled by other types of *R* genes. We report here that, under permissive environmental conditions, overexpression of *RPW8* under control of the native promoters, leads to H2O2 production, SA accumulation, increased expression of pathogenesis-related (*PR*) genes and HR-like cell death in absence of pathogens, and results in even higher level expression of *RPW8*, whereas under nonpermissive conditions, all the *RPW8* overexpression-mediated phenotypes and *RPW8*-meidated resistance are compromised, so is the increased expression of the *RPW8* genes. We therefore propose that enhanced expression of *RPW8* initiates defense-related programmed cell death (PCD) via a SA-dependent feedback amplification circuit, and that environment conditions may affect the *RPW8*-mediated resistance by tuning up or down the scale of this amplification. Additionally, we show that overexpression of *RPW8* (under permissive conditions), leads to enhanced resistance to virulent biotrophic pathogens but renders enhanced susceptibility to necrotrophic pathogens, suggesting pathogens with different modes of parasitism may differentially regulate host HR cell death.

6-51 bZIP factors TGA2 and TGA6 interact with SCRARECROW-LIKE protein 14 (SCL14)

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In higher plants, *activation sequence-1 (as-1)* of the Cauliflower Mosaic Virus 35S promoter mediates both salicylic acid- and auxin-inducible transcriptional activation. Originally found in viral and T-DNA promoters, *as-1*-like elements are also functional elements of plant promoters activated in the course of a defence response upon pathogen attack. *As-1*-like elements are characterised by two imperfect palindromes with the palindromic centres being spaced by 12 bps. They are recognised by the highly conserved TGA family of bZIP transcription factors. In electrophoretic mobility shift assays, bZIP protein TGA2.2 (a tobacco homologue of Arabidopsis factors TGA2, TGA5 and TGA6) binds efficiently to a mutant element carrying a 2 bp deletion between the palindromes *(as-1/(-2))*. However, this element is strongly compromised with respect to mediating gene expression in vivo. A fusion protein consisting of TGA2.2 and a constitutive activation domain mediates transactivation from *as-1/(-2)* demonstrating binding of TGA factors *in vivo*. We therefore conclude that transactivation requires optimal positioning of TGA factors on the *as-1* element. As TGA2 does not confer transcriptional activation in yeast, a co-activator was postulated. A modified yeast one hybrid system was established that used TGA2 bound to an *as-1* element as bait. Using an unfused *Arabdopsis* cDNA library a clone encoding protein "Scarecrow-like 14 (SCL14)" was isolated. In the presence of TGA2, this protein activates reporter gene expression only, if *as-1* is used as a *cis* element. Using *as-1/-2* as an upstream element, transactivation is severely impaired. Thus, SCL14 might function as a co-activator for TGA2-related transcription factors.

6-52 EDS1 and PAD4 are regulators of plant systemic immunity

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Systemic acquired resistance (SAR) is a plant immune response that is triggered after primary pathogen infection and provides long-term resistance to a broad range of pathogens. SAR requires salicylic acid accumulation (SA) and is associated with induction of various pathogenesis related (PR) genes. *EDS1* and *PAD4* are essential components of plant basal resistance against virulent pathogens and are recruited by a subset of *R* genes in race specific resistance. We have established that biological induction of SAR in Arabidopsis requires these two disease resistance regulatory genes, *EDS1* and *PAD4*. Both mutants exhibit hypersensitive response (HR) equivalent to wild type plants after inoculation with pathogens that trigger the EDS1-independent pathway, however in *eds1* and *pad4* plants the SAR response is completely abolished. This feature provides a suitable biological system to unravel the plant SAR response network after an intact local defence response. So the fact that they are also involved in SAR highlights their importance in plant defense signaling and may reflect the same activities. To understand the functions of EDS1 and PAD4 in the SAR pathway, we have analyzed expression of certain SAR reporter genes and performed several bioassays to evaluate the SAR response against different virulent pathogens as *Pseudomonas syringae* pv. maculicola ES4326 and *Peronospora parasitica* (Emco5). Also biochemical profiling of the wild type and mutant plant responses will be undertaken to identify consistent differences in metabolites and proteins upon triggering of SAR. Further approaches to unravel the roles of EDS1 and PAD4 in SAR signalling will be described.

6-53 Identification of regulators of Ics1 gene expression required for the defence response

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A salicylic acid (SA) biosynthetic pathway, previously identified in bacteria, has been found to be important for SA synthesis in Arabidopsis following pathogen attack. Isochorismate, which is a precursor of SA, is synthesized from chorismate by isochorismate synthase (*Ics1*). Plants that are mutated for *Ics1* are compromised for all modes of disease resistance (Nawrath et al., (1999) *Plant Cell* 11:1393-1404, Dewdney et al., (2000) *Plant Journal* 24:205-218). *Ics1* transcript is induced in response to pathogen attack and absent in unelicited plants (Wildermuth et al., (2001) *Nature* 414:562-565). Control of *Ics1* expression may therefore define an important regulatory mechanism for plant defence. A major goal of this work is to identify negative regulators of *Ics1*. I will mutagenize plants that have been transformed with an *Ics1*-promoter-luciferase fusion construct, and screen for mutants that show constitutive luciferase activity. A microarray experiment has been performed comparing the expression profiles of ics1 mutant plants with wild-type plants after infection with an avirulent strain of Pseudomonas syringae. This analysis has revealed that Ics1 is part of an Ics1-independent regulon. Genes with similar expression profiles to Ics1 will be monitored for their expression in known mutants as well as mutants identified by the Ics1-promoter-luciferase screen. A second more sensitive screen will be established by introducing the Ics1-promoter-luciferase construct into plants that express salicylate hydroxylase, which degrades salicylic acid. Transgenic seed will be mutagenized, and screened for constitutive luciferase activity. This screen may permit the identification of mutants that would ordinarily produce lethal levels of SA.

6-54 Role of *Atrboh* in defense response and programmed cell death

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Our goal is to address the functions of the Arabidopsis *Atrboh* NADPH oxidase *gp91phox* gene family, using functional genomics tools. The hypothesis to be tested is that members of this gene family control ROI (reactive oxygen intermediates) production during defense response and in several developmental contexts. We analyzed mutant lines deficient in the two highest expressed *Atrboh* genes, *D* and *F*. 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 electrolyte 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 weak avirulence on Col-0 wt. Paradoxically, although the *AtrbohD* contribution to total ROI production is greater than *AtrbohF*, individual mutant *atrbohF* displays the strongest effect on cell death. To further characterize the role of NADPH oxidase generated ROI in the hypersensitive cell death we performed epistasis studies between the *atrboh* mutants and the lesion mimic mutant *lsd1*. *lsd1* displays a runaway cell death in response to oxidative stress together with enhanced disease resistance. Interestingly, *lsd1* cell death is enhanced in the *atrboh* mutants, which suggests that ROI generated by the NADPH oxidase controls the spread of cell death in this lesion mimic mutant.

6-55 High-throughput reverse genetics: A potent mix of chips and GARLIC

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The completion of the Arabidopsis genome coupled with the availability of global expression profiling arrays has revitalized reverse genetics as a method of choice for functional genomic analysis. Reverse genetics is a strategy that proceeds from identification of interesting genes to mutations in those genes to phenotypic analysis of the mutants. Recently, TMRI and SBI have completed an ambitious reverse genetics project known as Gilroy Arabidopsis Reverse Library Insertion Collection (GARLIC). GARLIC comprises 100,000 individually isolated T-DNA insertion lines whose genomic regions flanking the T-DNA tag have been rescued, sequenced and compiled into a searchable database. We are using GARLIC to isolate knockouts in ~460 genes predicted to be involved in resistance to Pseudomonas syringae maculicola ES4326 based on extensive expression profiling. We have completed basic analysis of a survey set of ~100 genes for which we have confirmed the presence of the T-DNA insert and have biological data to determine the genes effect on resistance. These results will be presented along with our current strategy of high-throughput knockout isolation and phenotyping.

6-56 Defense-related gene expression in *Arabidopsis thaliana* triggered by the non-specific pathogen *Erwinia carotovora*

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The plant pathogenic enterobacterium *Erwinia carotovora* subsp. *carotovora* is the causal agent of soft rot disease. The virulence of the bacterium is correlated with its ability to produce and secrete plant cell wall degrading enzymes including pectinases, cellulases and proteases, which macerate plant tissue and release nutrients for bacterial growth. They are the main virulence determinants of the pathogen, but also have the ability to induce plant defense responses. In order to find new genes involved in the response to *E. carotovora*, we screened a subtracted cDNA library of Arabidopsis for *Erwinia*-induced genes. Culture filtrate (CF) containing cell wall degrading enzymes was used as an elicitor preparation. This screen resulted in isolation of more than 200 clones encoding proteins possibly involved in recognition of the elicitor or pathogen, signal transduction and production of antimicrobial molecules. Expression of the genes corresponding to the isolated clones was characterized in response to CF and exogenous application of plant signal molecules such as salicylic acid, ethylene and jasmonates. Some of the potential defense-related genes encoded WRKY DNA-binding proteins, protease inhibitors, lectin-like proteins, and proteins involved in oxidative stress. Over-expression and RNAi inhibition of these genes in transgenic Arabidopsis was used to determine their involvement in plant defense and will increase our understanding of the molecular mechanism of plant-*Erwinia* interaction.

6-57 Expression-profiling of genes of sulfur metabolism in response to pathogen stress

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The nutritional status of the plant has a strong impact on its tolerance towards pathogens. Sulfate deprivation is known to reduce the yield of crop plants of the brassica family due to fungal infections. To date the underlying mechanisms of such a "sulfur induced resistance" are not quite understood. In this project metabolite concentrations and gene expression patterns of primary and secondary sulfur metabolism are monitored under pathogen attack and in correlation to sulfur supply. To characterize the mechanism of sulfur mediated defense reactions the well established pathosystems *Arabidopsis thaliana* and *Alternaria brassicicola* as well as *Fusarium oxysporum* are investigated under sulfur limiting conditions. The function of sulfur-rich peptides in defense against fungal pathogens has been demonstrated before. In the genome of *Arabidopsis thaliana* four thionin isogenes are present. While two of them have been well characterized, the expression profiles of the other two isoforms have not been studied so far. Here we present a comparative analysis of thionin expression during plant development and in response to fungal infection. To monitor general changes of gene expression a macroarray is used that contains most key candidates for C-, N- and S-metabolism as well as for various stress-induced genes. As an important mediator of systemic signal transduction methyljasmonate is used in a first approach to trigger plant responses. A cluster analysis of the time course of gene induction after treatment of 4 week old Arabidopsis plants with 200 µM methyljasmonate was undertaken to identify responsive steps within the different pathways.

6-58 PDR5-like ABC transporters of *Arabidopsis thaliana*

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Plant pathogenic fungi, like various species of *Fusarium* and *Alternaria* produce mycotoxins in their host plants which play an important role in the development of plant diseases and are problematic for humans and animals when present in contaminated grain. Since the toxins most likely play a role as virulence factors, resistance against the fungal toxins should consequently lead to resistance against the producing pathogens. One group of candidate genes with a possible role in toxin resistance of plants are ABC transporters homologous to the yeast ABC transporter *PDR5*. Careful analysis of the available genomic sequences and ESTs revealed that the genome of *Arabidopsis thaliana* contains 15 *PDR5* like genes, for some of which the published annotation is questionable. In order to learn more about the substrate specificity of the *AtPDR*-genes we tried to express cDNAs in yeast mutants containing deletions of several ABC transporters. Unfortunately, even cDNAs fragments of some genes turned out to be toxic in *E. coli*. We therefore tried construction of plasmids allowing conditional expression of *AtPDR*-cDNAs by homologous recombination in yeast. Southerns and PCR results showed that the desired constructs were produced. Yet, we were unable to recover the yeast plasmid in *E. coli* in one case (AtPDR22), and the induction of the *GAL1* promotor lead to a tight growth defect of the transformed yeast in another case (AtPDR42). Due to these problems we now attempt regulated overexpression of the *PDR5*-like genes in plants.

6-59 A mutant screen for regulators of NPR1-independent resistance

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Systemic acquired resistance (SAR) is a state of enhanced resistance to a broad spectrum of virulent pathogens. SAR is associated with the increased expression pathogenesis-related (*PR*) genes and can be induced by a form of programmed cell death triggered by avirulent pathogens known as the hypersensitive response (HR). Mutants of *Arabidopsis thaliana* that are affected in SAR signaling have been identified through screens for constitutive expressors of pathogenesis-related (*PR*) genes (the *cpr*s) and non expressors of *PR* genes (*npr1*). The *cpr5* mutant spontaneously develops lesions which mimic the HR, and has constitutive resistance to virulent bacterial and oomycete pathogens, *Pseudomonas syringae* pv maculicola and *Peronospora parasitica* Noco2, respectively. The *npr1* mutant has enhanced susceptibility to these pathogens, and is unable to initiate SAR after either chemical or biological induction. The *cpr5npr1* double mutant is susceptible to *P. syringae*, but retains *cpr5*-mediated resistance to *P. parasitica*, suggesting that in the *cpr5* mutant both NPR1-dependent and NPR1-independent resistance pathways are activated. We have designed a screen in the *cpr5npr1* double mutant background for mutants that block NPR1-independent resistance. A population of 25,000 *cpr5npr1* seeds was fast-neutron mutagenized and infected with *P. parasitica* Noco2. Sixty-six independent lines were confirmed to be susceptible to *P. parasitica* and these have been classified according to defects in SA biosynthesis, ethylene sensitivity, jasmonic acid sensitivity, and *PR* gene expression.

6-60 The conserved effector locus in the Hrp pathogenicity island of *Pseudomonas syringae* pv. tomato DC3000 is required for disease in Arabidopsis and Tomato

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Pseudomonas syringae pv. tomato (*Pto*) causes disease in tomato and Arabidopsis and elicits the hypersensitive response (HR) in nonhost plants. Underlying the ability of the bacterium to cause these plant reactions is a functional Hrp (type III) secretion system, which appears to translocate effector proteins into plant cells. The *hrp/hrc* gene cluster is flanked by an Exchangeable Effector Locus (EEL) and a Conserved Effector Locus (CEL) in a pathogenicity island with a tripartite structure. We replaced *avrE*, a*vrF*, *ORF3*, *ORF4*, *hrpW* and *ORF5* (*hopPtoA1*) with an omega-SpR cassette to create a CEL mutation. The mutant no longer causes disease symptoms in tomato and Arabidopsis , has strongly reduced growth *in planta* and elicits a delayed HR response in tobacco. We identified and cloned a *hopPtoA1* homolog (*hopPtoA2*) located in a different region linked with transposase-like genes and *avrPphD* homologs. We demonstrate that HopPtoA1 and HopPtoA2 are secreted in culture by the type III secretion system and that mutation of *hopPtoA* genes reduce the ability of Pto DC3000 to initiate development of bacterial colonies in Arabidopsis leaves. Complementation analyses indicate that the CEL mutation can be complemented by a plasmid carrying *ORF3*, *ORF4* and a*vrF*. Mutation of *ORF3* causes reduction in virulence. We are using different approaches to better understand the role of *ORF3* during pathogenesis.

6-61 Potential quantitative nature of Arabidopsis responses during compatible and incompatible pathogen interactions

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Using Affimetrix oligo-arrays for >8000 Arabidopsis genes, we profiled mRNA expression during compatible and incompatible interactions between Arabidopsis and the bacterial pathogen *Pseudomonas syringae*. We examined incompatible interactions mediated by the resistance genes *RPS2* and *RPM1*, which correspond to the bacterial avirulence genes *avrRpt2* and *avrB*, respectively. We also observed effects of bacterial strain background difference (*P. syringae* pv. *tomato* DC3000 and pv. *maculicola* ES4326), effects of *ndr1* and *NahG*, and the response to a non-host pathogen *P. syringae* pv. *phaseolicola* NPS3121, in the mRNA expression profiles during the interactions. At a large scale of global profiling, we found that a single simple quantitative model could explain reasonably well differences between compatible and incompatible interactions, differential effects of *ndr1* and *NahG* on *RPS2*- and *RPM1*-mediated responses, and robustness in the system behavior. This finding suggests that major parts of the signaling mechanisms involved in compatible and incompatible interactions may be shared and that their differences in the responses may be mainly explained by differences in the signal flow in the common signaling mechanisms. The Arabidopsis response to *Psp* NPS3121 seemed similar to that observed during an incompatible interaction, but the amplitude was not as large and the kinetics seemed slower. Through these experiments we also identified genes whose expression changes are highly associated with a hypersensitive cell death. These are good expression markers for the hypersensitive cell death and good candidates for the genes functionally involved in induction of the hypersensitive cell death.

6-62 12 oxo-phytodienoic acid, the metabolic precursor of jasmonic acid as a signal molecule for plant defense responses

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Plant defense responses against insect herbivores and necrotrophic fungi rely on the biosynthesis and action of the signaling molecule jasmonic acid (JA). While most studies have focused on JA as the terminal product of the octadecanoid pathway, a signaling function has been proposed for its precursor 12 oxo-phytodienoic acid (OPDA), without prior metabolism to JA. Characterization of the Arabidopsis *opr3* mutant demonstrated a defect in the isoform of OPDA reductase required for JA biosynthesis. As a consequence *opr3* fails to metabolize OPDA to JA, but instead accumulates OPDA upon wounding. Unlike the Arabidopsis mutant *fad3-fad7-fad8* that lacks all JA precursors, or the *coi1* mutant impaired in JA perception, *opr3* displays wild-type levels of resistance to the dipteran insect *Bradysia impatiens* and to the necrotophic fungus *Alternaria brassicicola*. This indicates that resistance in *opr3* is mediated by a signal other than JA, the most likely candidate being OPDA. Analysis of transcript profiles in *opr3* showed that indeed, OPDA activates wound-responsive genes previously known to be JA-dependent, and that full activation of these genes through the COI1 signaling pathway involves the combined action of JA and OPDA. While OPDA is known to be synthesized in the chloroplast, evidence is presented here for OPR3 being co-localized with the final steps of JA biosynthesis in peroxisomes. The confinement to different subcellular compartments may contribute to the distinct signaling properties of the two molecules.

6-63 A screen for loss of non-host defense in Arabidopsis

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Plants are constantly exposed to a wide variety of pathogens. However, a given plant species is host to only a subset of these pathogens. Resistance to pathogens outside this subset is termed non-host resistance. Non-host resistance is thought to be multigenic, non-specific, and durable. In contrast, the resistance of certain genotypes of an otherwise susceptible species to a pathogen is termed host resistance. This type of resistance often follows gene for gene interactions and is typically short lived in the field. Isolating plant factors that affect non-host resistance may lead to a better understanding of non-host resistance. Arabidopsis is a host to the powdery mildew *Erisyphe cichoracearum*, and a non-host to *Blumeria graminis* f.sp. *hordei*, a pathogen of barley. A cytological comparison of host and non-host resistance showed that non-host resistance occurred early (1dpi) and correlated with the formation of papillae and strong callose accumulation. While most spores arrested at penetration, 6% of non-host spores formed haustoria. In contrast, host resistance occurred late, with callose deposition only at the papillae. 12,000 EMS-mutagenized plants were screened for altered callose response to the non-host mildew. Two classes have been further studied: plants exhibiting increased callose deposition around penetration events, and plants with a high frequency of fungal penetration events. Twelve lines that exhibit increased penetration fall into four different complementation groups. Complementation is underway for the classes that exhibit increased callose deposition. The response to wounding, Peronospora and Phythopthora inoculation, as well as mapping data, will be presented.

6-64 Identification of systemic acquired resistance-independent disease resistance

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A major goal of current research in molecular plant pathology is to elucidate the range and capabilities of pathogen-induced defense systems that plants use to prevent and recover from infection. Systemic acquired resistance (SAR) is one such system, which requires for induction the accumulation of salicylic acid (SA) and activity of the *NIM1/NPR1* gene. Plants unable to express SAR show substantial defects in their resistance phenotype, but importantly also still express significant residual resistance, indicating that in these plants, SAR-independent resistance (SIR) is both exposed and accessible to molecular genetic analysis. We have taken several approaches to exploit this system by seeking mutants that are unable to activate or are constitutively activated for SIR. I will discuss our results toward elucidating SIR pathways, emphasizing the discovery, characterization and cloning of the Arabidopsis *SON1* (*SUPPRESSOR OF NIM1-1*) gene, identified as a recessive mutation that confers strong resistance to *Peronospora parasitica* in the SAR-incompetent *nim1-1* mutant. *SON1* encodes an F-box protein, implicating the regulation of *son1*-mediated resistance through a mechanism involving targeted ubiquitination and destruction of a positive regulator of SIR. Because resistance in *son1* is not linked to SAR gene induction, and is expressed in SA-depleted and *nim1-1* mutants, *son1* plants express an SIR response. In other work, we manipulated expression of the NIM1/NPR1-interacting transcription factor TGA5, and found the transgenic plants to display strong resistance against *P. parasitica* and bacterial pathogens without evidence of molecular markers of SAR or requirement for SA or NIM1/NPR1, also implicating an SIR response.

6-65 The mutant *Verticillium hypersensitive* 1 identifies a new Arabidopsis locus connecting the control of the transition to flower to both biotic and abiotic stress responses

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The mutant *Verticillium hypersentive* 1 was isolated in a genetic screen for increased sensitivity to the soil-borne fungal pathogen *Verticillium dahliae* Kleb. The inoculation of the root system of two-week old seedlings resulted in the complete bleaching of the rosette leaves within two weeks from the infection, whereas, in C-24 wild type, chlorosis was limited to the cotyledons. The *vhs* 1 allele is semi-dominant and in addition to *Verticillium* tolerance affects development and salt stress response. *vhs* 1 exhibits an early flowering and semi-dwarf phenotype that is sensitive to photoperiod, with a more severe effect of the muration occurring under in long day conditions. The analysis of trichome distribution revealed the absence of adult leaf formation and the direct transition from juvenile to reproductive phase in long day photoperiod. No detrimental pleiotropic effects of the mutation on floral organ development or fertility were detected. Remarkably, treatment with NaCl of six-day old seedlings caused in *vhs* 1 a delay in the transition to flower compared to untreated plants and an increase in NaCl tolerance compared to wild type plants. *vhs* represents a unique genetic background for studying interactions between processes regulating life span and stress responses.

6-66 Arabidopsis as model plant for studies of resistance to powdery mildews

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Plants resist attacks from powdery mildew fungi either at the stage of penetration, when papillae are formed in the host epidermal cell wall, or by a hypersensitive cell death reaction (HR) at a later stage. These cellular responses also occur when Arabidopsis exhibits non-host resistance to the barley powdery mildew fungus (Blumeria graminis f.sp. hordei, Bgh). Most conidia are arrested at the stage of penetration, and clear papilla formation occurs. Nevertheless, some conidia penetrate and develop functional haustoria. Within 2 days after inoculation, the individual penetrated epidermal cells undergo HR. A microscope-based screening for Arabidopsis mutants with altered responses to Bgh was conducted on M2 lines of EMS-treated Columbia. Two mutants were identified with higher penetration rates and therefore higher numbers of single cell HRs. These increases in HR are detectable by the naked eye. Intermutant crosses have demonstrated that the mutations occur in the same gene. These mutant alleles are named pen1-1 and pen1-2 for higher penetration rates. Histochemical analyses of papilla of pen1-1 plants reveal no obvious alterations in the content of callose, structural protein or H2O2. Knowing that pen1-1 and pen1-2 plants have a hampered resistance, it was surprising to learn that they have an increased level of transcripts for pathogenesis related (PR) protein. In order to map-base clone PEN1, this gene has been mapped to a ca. 50 kb sequenced interval with 15 genes. A suppressor mutant screen is currently being conducted, and results from this will be presented.

6-67 Analysis of global gene expression in *hxc2*, an Arabidopsis mutant altered in the hypersensitive response to the bacterial pathogen, *Xanthomonas campestris* pv. *campestris*

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Xanthomonas campestris pv. *campestris* (*Xcc*) is the causal agent of black rot on cruciferous plants and a natural pathogen of *Arabidopsis thaliana*. Resistance of Arabidopsis accessions Col-0 and Ws-4 to the strain 147 of *Xcc* is determined by a single dominant locus and accompanied by the hypersensitive response (HR), a rapid and localised cell death at the infection site. By screening an EMS-mutagenised seed library of Col-0 plants, we identified mutants displaying alterations in the HR in response to *Xcc* 147 infection. One mutant, *hxc2* (for hypersensitivity to *Xcc*) is susceptible to *Xcc* 147, as shown by measurement of *in planta* bacterial growth. The mutation causes pleiotropic alterations of defence responses and acts upstream of salicylic acid accumulation (Godard et al., 2000). Genetic analysis showed that the *hxc2* mutation is inherited as a monogenic recessive trait, different from the *R*-gene involved in the recognition of *Xcc* 147. However the resistance to a majority of other bacterial or fungal pathogens is not affected in *hxc2*. In an effort to gain an overview of the multiple downstream genes related to the *hxc2* phenotype, we have used cDNA microarray technology to profile the expression pattern of over 8000 Arabidopsis genes. Sampling of time points during the first 24h after inoculation with *Xcc147* revealed significant changes in the steady state transcript levels of approximately 500 genes between the wild type and the *hxc2* mutant. The differentially expressed genes are involved in many cellular processes and a more detailed analysis of a subset of them is under study.

6-68 *svn1*, an Arabidopsis mutant affected in programmed cell death: phenotypical analysis and characterization of the corresponding gene

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In plants, one of the most efficient resistance reaction to pathogen attack, is the so-called Hypersensitive Response (HR) characterized by a programmed, rapid and localized cell death at the inoculation site. To identify and characterize genes involved in the regulation of HR-related cell death programmes, one powerful approach is to look for mutants showing spontaneous "HR-like" lesions in the absence of any pathogen (lesion mimic mutants). By screening a T-DNA mutagenized seed library of Arabidopsis, we identified such a mutant, *svn1* (for spontaneous vessel necrosis), displaying necrotic HR-like lesions, propagating along the vascular system. The mutation has been shown to be light conditionning, tagged monogenic and recessive. An other mutant allele displays a similar phenotype. In addition, complementation test clearly demonstrated that *SVN1* is implicated in lesion development. *SVN1* is located on top of chromosome 1 and encodes an unknown protein with structure organization similar to proteins from Arabidopsis, *C. elegans* and mouse. In response to pathogens, under lesion "+"conditions, *svn1* exhibits enhanced susceptibility to avirulent and virulent isolates (*Xanthomonas campestris* pv *campestris*). Interestingly, constitutive expression of defense genes (*PR1, PAL, PDF1.2*) and production of high level of SA are observed in the mutant in absence of pathogens. In lesion"-"conditions, the mutant behaves at the wild type, or shows enhance resistance, depending on the pathogen tested. Using a promoter-GUS fusion, *SVN1* expression was shown localized at the vicinity of the hypersensitive lesions suggesting a role in cell death limitation. The existence of a new protein in plants, involved in the control of cell death, will be discussed.

6-69 Cross talk of defense pathways in arabidopsis infected by the whitefly *Bemisia tabaci* and the root-knot nematode *Meloidogyne incognita*

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Plants use constitutive and induced defenses against pathogen and herbivore attack. The mechanisms that control the plant responses are very complex, and depend on the type of the interaction. In general, the signalling pathways activated are related to the accumulation of the salicylic acid (SA), jasmonic acid (JA) and ethylene, as well as, the induction of the systemic acquired resitance (SAR), that leads to a broad range of resistance mechanisms against diverse pathogens. The majority of data have been taken from studies with pathogen and herbivore that produce damage of plant tissues. Recently few groups are working on interactions without wounding effects, trying to prove the accepted signal pathways. Phloem-feeding whiteflies and root-knot nematodes produce little injury to plant tissues, since they introduce their stylets and keep the contact with the cell during their development. Both organism have been choose in this work, in order to characterize the interaction wiht Arabidopsis, allowing us to detemine the activation of responses pathways and the hint of SA, JA or the SAR induction. Besides, the infection of several Arabidopsis mutants, deficients in part of the defenses pathways, will help us to complement the defenses pathways proposed.

6-70 Biological activity of recombinant extracellular protein 2 (ECP2)

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The interaction between the hemibiotrophic fungus *Cladosporiumfulvum* and its host tomato (*Lycopersicon esculentum*) obeys the classical gene-for-gene relationship. Fungal growth is restricted to the apoplastic space of tomato leaves, where *C. fulvum* secretes small, cysteine-bridged avirulence (AVR) proteins. AVR proteins elicit anisolate-specific resistance response in tomato genotypes with the corresponding resistance (*Cf*) genes. The AVR proteins that are secreted by all *C. fulvum* isolates and that contribute to fungal virulence are called extracellular proteins (ECPs).ECP2 has a role in (a)virulence in tomato and is recognised in certain non-host *Nicotiana* species (Laugé *et al*., *PlantJ*. 23: 735).Our goal is to identify the function of ECP2 in establishing or arresting infection in tomato and non-host plants (*Nicotiana* sp., *Arabidopsis thaliana*, etc.). ECP2 was traditionally! isolated from intercellular wash.

6-71 New insights into the possible role of the NIMIN proteins in systemic acquired resistance

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The NIMIN proteins were recently found in a screen for proteins interacting with NPR1 (aka NIM1), a key regulator of systemic acquired resistance (SAR) in plants. NIMIN-1, NIMIN-2 and NIMIN-3 were shown to interact strongly and specificly with NPR1 in the yeast two-hybrid system and *in vitro*. *NIMIN*-mRNA levels are transiently elevated after SAR-inducing stimuli suggesting a possible role of the NIMIN proteins in SAR. Despite low overall similarity among the NIMIN proteins they share some common domains identifying them as members of a small protein family in *Arabidopsis thaliana*. To further analyze whether the interaction with NPR1 is part of the signal transduction cascade leading to SAR we have studied the interaction of NIMIN, NPR1, TGA factors and DNA in yeast. Members of the TGA family of transcription factors are also known to bind to NPR1 and are involved in SA-mediated gene expression in SAR. Transient assays with BY-2 protoplasts indicate that the NIMIN proteins might be involved in gene regulation. Taken together, we suggest that the NIMIN proteins are part of the regulatory network enabling differential SAR responses in challenged Arabidopsis plants.

6-72 Genetic analysis of susceptibility to *Peronospora parasitica* in Arabidopsis

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Plants are susceptible to a limited number of pathogens. They resist most infections by early pathogen recognition and the subsequent activation of plant defense responses. To grow and reproduce on plants and to avoid recognition, pathogens have evolved advanced mechanisms to attack host cells. The oomycete pathogen *Peronospora parasitica* interacts with the plant through haustoria, feeding structures that invaginate host cells but remain surrounded by a host membrane. To gain insight into the genetic basis of disease susceptibility and haustorium function, Arabidopsis
EMS-mutants were created in the *eds1-2* background. From the parental *eds1* (for enhanced disease susceptibility) line, that supports abundant pathogen growth and sporulation, resistant mutants were isolated on which no *Peronospora* sporulation was visible. In most of the *dmr* (for downy mildew resistant) mutants, isolated so far, *Peronospora* growth is arrested or retarded. Microscopic analysis showed that in many cases *Peronospora* haustoria are surrounded by callose and/or have an aberrant form, indicating a distortion of the plant-pathogen interaction. Most known mutants with enhanced pathogen resistance show induced activation of defense responses. The majority of *dmr* mutants, however, do not show induced defense gene expression or resistance to other pathogens, e.g. *Pseudomonas syringae*. We hypothesize that important cellular targets for pathogen infection are disrupted in these mutants. We will report on the chromosomal map position of three *dmr* mutations and our effort to clone the corresponding *DMR* genes. Their functional analysis will provide new insights into disease susceptibility and the molecular processes that occur at the host-pathogen interface.

6-73 Defining the host transition from innate to resistant response

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In some race specific interactions a highly evolved surveillance mechanism operates to recognises the presence of a specific type III effectors, resulting in a massive reprogramming of transcription and ultimately disinfection of the invading host. Although pathogen transcriptional reprogramming is well documented the temporal engagement of host genes during the course of an infection necessitates a well-characterised pathosystem. In the Arabidopsis *avrRpm1/RPM1* interaction, key landmark events associated with avirulence gene induction and increases in intracellular calcium through to leaf collapse can be assayed over a time scale of 5h. Using this interaction we examined the fundamental transcriptional networks underlying both basal and induced defence responses through gene expression profiling by AFLP-cDNA. We demonstrate a rapid dynamic host response to pathogen invasion, presumably through a highly conserved innate recognition of pathogen associated molecular patterns. Changes in the steady state levels of a broad range of transcripts are detected within 30 min. of bacterial challenge, peaking between 1-2h. This was prior to *in planta* detection of *avrRpm1* and consistent with the inability to discriminate between DC3000 *hrpA* and DC3000 treatments on host transcriptome reprogramming during the early stages of infection. As the resistant interaction progressed, novel *R* gene specific fingerprints were identified within 30 min. of maximal cytosolic calcium increases, 2h prior to any macroscopic pathological responses. THe uniqueness of the interaction is underlined by the fact 60% of the differentials identified are not represented on the current Affymetrix Gene Chip set.

6-74 RAPers required for resistance; Components of the RPM1 signalling network

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We have begun dissecting plant disease resistance signalling networks recruited by RPM1, a member of the largest class of plant disease resistance genes, containing both a nucleotide-binding domain and leucine-rich repeats (NBS-LRR). To this end we used the apoptotic ATPase domain (also known as NB-ARC or NOD domain) of RPM1 as bait to screen a 2-hybrid prey library constructed from pathogen induced tissue. Here we describe two genes, *RAP12* and *RAP23* (*R*PM1 *A*poptotic ATPase interacting *P*rotein) identified in this study. Transgenic plants ectopically expressing *RAP12* or *RAP23* significantly delay or abolish hypersensitive cell death elicited by challenge with bacteria expressing *avrRpm1*. Both lines are hyper-resistant, with bacterial growth significantly restricted compared to wild type plants. Antisense of either *RAP* gene significantly enhances bacterial growth in the presence of *avrRpm1*. Additionally, antisense *RAP12* plants result in an accelerated HR in the presence of *avrRpm1*. Critically, all sense and antisense lines of *RAP12* and *RAP23* are phenotypically normal, with no indication of general metabolic perturbation, nor induction of markers indicative of activated defense responses such as *PR1*. In all transgene lines the *avrRpt2*/*RPS2* interaction results in identical timing of the HR, and the restriction of bacterial growth as seen in wild type plants, indicating no enhancement of basal defense responses. We hypothesize RAP12 and RAP23 cannot be proteins guarded by RPM1 as over-expression results in enhanced, rather than loss of RPM1 function. We will further describe the RAP genes and ongoing functional studies on their interaction with RPM1.

6-75 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*). While wild-type plants are fully susceptible and normally don't develop lesions in response to powdery mildew, these mutants are resistant to the pathogen and abnormally begin to form necrotic lesions four to seven days after inoculation. By cloning and characterizing the *mil* mutants I hope to discover novel components of the signal transduction pathways leading to defense responses, especially cell death. The mutants fall into at least seven complementation groups that display a range of cell death, chlorosis and resistance phenotypes. Three recessive mutants that form induced lesions specifically in response to pathogens are being pursued for further characterization and positional cloning. So far, these three mutants have been mapped to small intervals on chromosomes three and four. The resistance conferred by these mutations is late acting, allowing the pathogen to invade and grow to a limited extent, but blocking the later asexual reproduction. These mutants also develop similar lesion and resistance phenotypes in response to *Peronospora parasitica*, another obligate biotrophic pathogen.

6-76 Proline Extensin-like Receptor Kinase (PERK) family in Arabidopsis

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Plant receptor kinases form one of the most abundant protein classes able to sense changes at the cell surface and initiate appropriate responses through signal cascades. The *PERK* family in *Arabidopsis thaliana* consists of 14 genes identified by amino acid sequence similarity to *Brassica napus PERK1*, a novel receptor kinase that appears to play a role in the wound response and in plant development (Silva & Goring, Plant Mol Biol, in press).
Characterization of this family in Arabidopsis by RNA gel blot analysis shows that the *PERK* family members are differentially expressed during development. *AtPERK1*, which shares high similarity with *BnPERK1*, is proposed to represent the PERK1 orthologue in *A. thaliana*. The mRNA level of *AtPERK1* increases dramatically upon wounding as does that of *BnPERK1*. The expression pattern of *AtPERK1* and other members upon wounding and infection with *Pseudomonas syringae pv tomato* (*Pst*) using RNA gel blot analysis and promoter-GUS transgenic plants will also be presented.
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6-77 Age-related resistance: a novel, developmentally induced defense response that utilizes the signaling molecule, Salicylic Acid

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Age-related resistance (ARR) is a form of resistance that develops in mature *A. thaliana* plants in response to *Pseudomonas syrigae* (*Pst*) infection. The salicylic acid (SA)-deficient lines NahG, *sid1*, *sid2*, and *pad4* demonstrate the importance of SA accumulation during ARR by their inability to display this defense response. Previous studies demonstrate that PR-1 gene expression does not correlate with ARR and that ARR is a distinct response in that it occurs in a number of *Arabidopsis* defense response mutants (*npr1*, *pad3-1*, *eds7-1*, *etr1*) (Kus *et al.*, Plant Cell 14 2002, pg. 479-490). Intercellular washing fluids (IWFs) from plants expressing ARR exhibit anti-bacterial activity to *Pst* and SA does accumulate in IWFs from plants expressing ARR, suggesting that SA may act as a signal for ARR-associated defense or it may itself possess anti-microbial activity.

6-78 Molecular genetic dissection of the RPP7 resistance pathway

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The *RPP7* gene activates race-specific resistance to the downy mildew pathogen *Peronospora parasitica*. Previously published genetic epistasis tests have established that *RPP7* activates defense responses through a signaling mechanism that does not require accumulation of salicylic acid (SA) or components of the ethylene and jasmonic acid response pathways encoded by *EIN2*, *JAR1*, or *COI1*. Furthermore, *RPP7* is not suppressed by mutations in a variety of putative signal transducers that are required by various NBS-LRR resistance genes (e.g. *pad4-1*, *ndr1-1*, *npr1-1*, *pbs2-1*). In an effort to better understand the genetic requirements for signal transduction from RPP7, we have constructed a series of double mutants to test for additive or functionally redundant contributions by known defense signaling components. We observed that *RPP7* is partially suppressed in *pad4-1*/*ndr1-1*, *pad4-1*/*npr1-1*, pad4-1/*pbs2-1*, *ndr1-1*/*pbs2-1*, and *ndr1-1*/*npr1-1* backgrounds. These results reveal "cryptic" roles for PAD4, PBS2, NDR1, and NPR1 in RPP7 signaling, and suggest that RPP7 activates resistance through multiple, functionally redundant signaling pathways. We are completing an effort to clone the *RPP7* gene, using map-based methods. This gene has been mapped to a large cluster of CC-NBS-LRR genes on Chr.1, and we are currently determining which of these candidate genes encodes *RPP7*.

6-79 Investigation of the host-virus interactions using *Arabidopsis* T-DNA insertion lines

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Transcriptional profiling and reverse genetic analyses have been utilized to examine global gene expression and function in virus-infected *Arabidopsis* plants. Results from the transcriptome study identify more than 1,000 genes that are induced or suppressed during the virus infection course. Some of these genes, such as heat shock proteins and glucanases, have been reported to be involved in host defense response. However, most of the genes have not been correlated with virus infection. In addition, some genes have not been described or characterized. The 1,000 genes from the profiling analysis are grouped based on their potential functions, and several gene families are selected for further investigation. These targeted groups will be examined for their potential roles in virus pathogenicity or host defense using *Arabidopsis* T-DNA insertion lines. This reverse genetic approach provides us insight of plant-virus interaction and characterizes function of unknown genes and proteins in development as well as in defense system.

7-01 Control of cell proliferation and differentiation by the D-type cyclin CYCD3

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CYCD3;1 expression is associated with proliferating tissues such as meristems and developing leaves but not with differentiated tissues in Arabidopsis. Constitutive overexpression of *CYCD3;1* in cultured cells and in shoot apices leads to a shift of cells from G1 (2C) to G2 (4C) DNA content. In transgenic plants development is retarded, and leaf architecture is radically altered, with a failure to develop distinct spongy and palisade mesophyll layers. We observe hyperproliferation of leaf cells, and in particular the epidermis consists of large numbers of small, incompletely differentiated polygonal cells. As a consequence, cell division largely replaces cell expansion as the primary mechanism for leaf growth, although the expression of *AINTEGUMENTA* which is proposed to control leaf cell number is unchanged. Other defects in leaf and stem tissues are consistent with the continued proliferation of many cell types preventing their complete differentiation. Endoreduplication, a marker for differentiated cells that have exited from the mitotic cell cycle is strongly inhibited in *CYCD3;1* overexpressing plants. These results demonstrate that cell cycle exit in the G1 phase is required for normal differentiation processes during plant development, and show that CYCD3;1 controls cell proliferation and cell differentiation in leaves likely acting downstream of *AINTEGUMENTA*.

7-02 PDK1-dependent lipid-signalling pathways in Arabidopsis

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Identification of key genetic factors controlling plant growth is a long-term goal, both for plant breeding and fundamental plant science. PDK1 has attracted considerable attention, as it is a central integrator for signalling events from receptors that stimulate PI 3-kinase and regulate a number of processes, the most prevalent of which is to maintain the balance between growth, cell division and apoptosis in animals. Although plants do not produce PtdIns(3,4,5)P3, the lipid-activator of this pathway, a functional homologue for PDK1 has been found and was shown to bind a wide range of lipids. To find further PDK1-targets in plants we performed an interaction screen, and thus identified three AGC class-protein kinases. Reducing AtPDK1 abundance through RNAi constructs only affected the activation of some of these kinases, establishing downstream links within the lipid-signalling pathways in plants. Having found candidates for downstream target of AtPDK1 in plants, we investigated the lipid activators of AtPDK1 signalling by assaying AtPDK1-dependent activation. Amongst a set of phospholipids, added to plant cells, only some stimulated these kinase activities and it was dependent on the presence of AtPDK1. Most notably, the two prevalent plant growth-stimulating hormones synergistically stimulated the pathway. These experiments provide the first sketch for a lipid-signalling pathway mediating growth hormones in plants.

7-03 Expression of *HOBBIT* and *AtCDC27a*, two homologues of an APC component

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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 in the basal part of the embryo. However, post-embryonicaly, *hobbit* mutants display several complex phenotypes, such as defective meristems and absence of specific cell identities. This suggest that HOBBIT function is involved in controlling cell division patterns as well as acquisition of some cell fates. The *HOBBIT* gene has been cloned and sequences analysis suggests homology with CDC27, a TPR-containing protein involved in the Anaphase Promoting Complex. A second CDC27-related sequence is present in the arabidopsis genome. The deduced AtCDC27a protein shares a homology of 41% with HOBBIT. To determine the expression pattern and subcellular localization of both proteins, a hemaglutinin tag has been introduced in the *HOBBIT* genomic rescuing fragment at the C-terminal part of the protein. We have also generated an antibody against a divergent region of AtCDC27a.
Hobbit mutants display a very drastic post-embryonnic phenotype. To circumvent this pleiotropy in phenotype and distinguish between roles in cell differentiation or/and cell division for HOBBIT, we are performing a clonal analysis by generating *HOBBIT* mutant sectors in phenotypically wild type plants. Preliminary results of this clonal analysis will be presented.

7-04 *In planta* analysis of SHOOT MERISTEMLESS (STM) protein functions and isolation of potential interaction partners in the shoot apical meristem of *Arabidopsis thaliana*

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By use of the Chimeric Repressor Interference System (CHRIS) the KNOX-ELK domain of the STM protein was identified as the minimal sequence element needed to trans-dominantly phenocopy *stm* loss-of-function alleles in transgenic *Arabidopsis* plants. Clear phenotypic differences between plants overexpressing the KNOX domain (squelching) and a chimeric *en* -KNOX repressor fusion, suggest that the KNOX domain is efficiently integrated into multi-component protein complexes and presumably recruited to the DNA, where the chimeric *en* -KNOX protein represses target genes. The specificity of detectable phenotypes in transgenic KNOX or *en* -KNOX plants in the SAM indicates, that essential partner proteins in *Arabidopsis* may also be confined to the SAM. To isolate genes encoding such protein partners, a meristem specific yeast two-hybrid library was established and screend for interacting proteins. Independent clones of several transcription factors belonging to different families (ERF, bHLH, SUP-like, ARF-like and several HD-classes) were isolated. The specificity of protein interactions has been confirmed in yeast and compared between the *KNAT1* and *STM* KNOX domains. While several proteins interact with both KNOX domains others exhibit specificity. Presently we are in process to analyse the cellular expression patterns of candidate genes by performing RNA *in situ* hybridisations. First results either show overlapping patterns with *STM* or expression domains confined to the SAM.

7-05 Traitmill^{TM}: A high throughput platform for phenotyping transgene effects in plants

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CropDesign technology platform closes the application gap between classical genomics and the development of improved or novel crop traits. Trait development requires testing the effects on plants of varying expression levels of thousands of genes and gene combinations involved in complex and valuable traits. CropDesign Traitmill TM has beenspecifically designed for this purpose.

i) Using a high throughput proprietary gene cloning system, expression levels of genes and gene combinations can be modulated throughout entire plants or in selected tissues.
ii) An industrialized plant transformation system generates thousands of transgenic plants annually to analyse the gene andgene combination leads.
iii) Automated evaluation technologies, including digital image analysis of transgenic plants, are employed to generate the large volume of trait-specific data required for product development.

The Traitmill TM employs rice and Arabidopsis for high throughput gene testing. Cropdesign biology research focuses ongenes modulating size, shape and growth of specific plant tissues, and plant as a whole, but also on interactions with theenvironment via multiple signal exchange networks, and on production, uptake, translocation and sensing of nutrients. Many of the genes controlling these complex processes remain functionally undefined and are the target of functional genomics research at CropDesign. Cell cycle is the mechanism that regulates cell division, the fundamental means by which organisms grow and propagate. CropDesign has observed different phenotypes with modified expression of cell cyclegenes, including enhanced growth, overproliferation of leaf organs, dwarfism and anthocyanin accumulation. A phenotypic description of these transgenic lines will be presented.

7-06 Chromatin behavior and interlocus distance in *Arabidopsis thaliana* guard cells

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In spite of increases in the database for plant genomes, our understanding of how DNA is organized in the plant interphase nucleus remains poorly developed. Recent applications of a novel technique that allows direct visualization of tagged loci with fluorescent proteins in the interphase nucleus of yeast and in vitro cultured animal cells have shown that chromosomes can undergo constrained diffusive motion. Furthermore, "trans-association" between two inserted concatameric arrays has been observed in yeast using this technique. We extend these observations to intact Arabidopsis seedlings for visualization and tracking of specific insertion sites. Focusing our analyses on nuclei of differentiated guard cells in a transgenic line that contains two tagged loci separated by 4.2 megabase-pairs, we find evidence for constrained motion as well as significant trans-association. The two unassociated loci in hemizygous plants are found to be located 1.10 micron apart with little variance between nuclei of individual guard cells. This suggests that there may be preferential order of interphase chromatin at the megabase-pair scale in mature guard cells.

7-07 Interactions between the PTS1 and PTS2 protein import pathways in plant peroxisomes

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Peroxisomal matrix protein import requires two cytosolic receptors. Pex5p, the PTS1 receptor and Pex7p, the PTS2 receptor, recognize different peroxisomal targeting signals (PTSs). Though the PTS1 and PTS2 receptors seem to define separate import pathways, recent experiments suggest that there is a convergence between the two pathways. Two isoforms of *PEX5*, created by alternative splicing of the mRNA, exist in mammals. The long form, but not the short form, has been shown to interact with human Pex7p. This interaction has not been observed, however, in yeasts or in plants. Arabidopsis *PEX5* (AtPEX5) more closely aligns with the long form of mammalian *PEX5* genes than with yeast *PEX5*, suggesting that AtPex5p may interact with Pex7p during matrix protein import. To test this hypothesis, we used yeast two-hybrid studies and protein blot overlay assays. Results from these experiments suggest that AtPex5p does indeed interact with Pex7p from tomato. Furthermore, utilizing in vitro peroxisomal protein import assays, we directly investigated the effect of varying components in one import pathway on the import of proteins along the other pathway. The addition of a PTS1 protein stimulated the import of a PTS2 protein several fold. Similarly, addition of a PTS2 protein clearly stimulated the import of various PTS1 proteins. Finally, depleting Hsp90 from an import reaction significantly inhibited the import of a PTS2 protein, although Hsp90 appears to interact directly with the PTS1 receptor, Pex5p. Taken together, these data support a model for plant peroxisomal matrix protein import in which the two pathways directly interact.

7-08 AtE2F2, a cell cycle gene repressor, is regulated by ubiquitin-mediated degradation

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Selective ubiquitin-mediated proteolysis through the cell cycle controls the availability, and therefore the activity, of several proliferating factors. E2F transcription factors regulate the expression of cell cycle and differentiation genes depending on their interaction with RB proteins. The components of RB/E2F pathway, as well as other cell cycle regulators, have been identified in plants. In this work, we will present data that support that Arabidopsis E2F2 is regulated by the ubiquitin-proteasome pathway, implicating the function of the E3 ubiquitin-ligase SCF-AtSKP2. Furthermore, phosphorylation of AtE2F2 by an AtCDC2a/CycA complex is required for interaction with the F-box protein AtSKP2. Interestingly, the auxin response mutant *axr1-12*, in which the modification of CUL1 with RUB1 is impaired, shows increased AtE2F2 protein levels, suggesting a dysfunction in the control of AtE2F2 stability. In addition, expression of a truncated AtE2F2 into the *axr1-12* partially restores the mutant phenotype.
To understand the role of AtE2F2 we generated transgenic plants that express a truncated AtE2F2, lacking the regulatory N-terminal region. Preliminary results show that both the cell shape and cell length are affected in these transgenic plants. We also found that some cell cycle genes containing E2F-site in their promoter are down-regulated. In vitro assays showed that AtE2F2 directly interacts with a plant RB and, interestingly, such interaction is increased by the presence of DNA containing an E2F-site. Taken together, these data suggest that AtE2F2 functions as a gene repressor of cell cycle genes and its availability is regulated by ubiquitin-mediated proteolysis.

7-09 *ORC* gene expression, required for DNA replication, is regulated by E2F transcription factor

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Cell cycle is a highly regulated process where transitions through different phases depend on checkpoints. One of them, the G1/S checkpoint, is controlled by the RB/E2F pathway. Before the onset of S phase, E2F activity is released and able to induce the expression of a number of genes required for S phase.
Members of the pre-replicative complex (ORC, CDC6, CDT1 and MCMs) are required for DNA replication. At the onset of S phase the activation of the pre-replicative complex allows the initiation of replication. The function of this pre-replicative complex is to mark and activate the origins of replication in S phase in a coordinated way, because each origin must activate once and only once per cycle.
It seems that the basic mechanisms of origin activation is common in all the metazoa. However there are very important differences at the level of regulation and information is lacking in plants. Here, we report the identification and cDNA isolation of all the six components of the ORC complex in Arabidopsis. All of them have conserved domains, although in some cases the homology is rather limited. Unexpectedly in Arabidopsis there are two ORC1 genes, which share 91% homology at nucleotide level. Expression of ORC genes is cell cycle regulated, and interestingly most of them have E2F binding motifs in their promoters. This suggest an E2F regulation of the ORC complex expression in plant cells. The implication of this regulation will be discussed.

7-10 The DNA replication initiation factor AtCDT1: CDK phosphorilation and effects of ectopic expression

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The tight regulation of DNA replication is crucial for the maintenance of cellular integrity. A pivotal role for this regulation, as far as it is known in other systems, is the availability of the different proteins that form the pre-replication complex, that is the case of ORC, CDC6 and CDT1. Although homologs for these proteins have been described in different model systems, the regulation of these proteins during the cell cycle seems to diverge among them. This justifies this study in Arabidopsis, an eukaryotic model system with unique characteristics of growth and development. We are interested in the study of the regulation of the pre-replicative complex in Arabidopsis, mainly in the regulation of the components CDC6 and CDT1. After the characterization of AtCDC6 and the study of some aspects of its regulation (Castellano et al., 2001), we have isolated two genes encoding AtCDT1 proteins. They share low sequence homology with the CDT1s described in other system but they possess conserved domains within this family. Indeed, both proteins interacts with AtCDC6a. In this work we have studied the phosphorylation of both AtCDC6a and AtCDT1a by differents CDK/cyclin complexes, and found that these proteins interact with the CDK/cyclin components both *in vitro and in vivo*. The potential roles of this modification will be discussed. We will also show the effects of overexpressing AtCDT1 in Arabidopsis transgenic plants.

Castellano, M.M., Pozo, J.C., Ramirez-Parra, E., Brown, S. and Gutierrez, C.(2001) "Expression and stability of Arabidopsis CDC6 is associated with Endoreplication". The Plant Cell, 13: 1-17.

7-11 Identification and cell cycle regulation of E2F target genes

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E2F/DP transcription factors are one of the most important regulators of the G1/S transition of the cell cycle. In addition, they are implicated in others process such as differentiation and development. However, the direct targets of this transcription factor remain unknown. The completion of the Arabidopsis genomic sequence offers the possibility to extract global information about regulatory mechanisms that govern cell and organism organization. Here, we describe a strategy to mine the raw Arabidopsis genome sequence and identify *bona fide* genes directly regulated by E2F/DP.
Starting with a genome-wide search of chromosomal locations containing E2F binding sites, we studied in depth two of the most abundant E2F binding sites within the Arabidopsis genome and identified over 180 genes containing these sites in their promoters. We analized other parameters crucial for funcionality of these sites, such as relative distance of the sites in the promoters or presence of other sites in its close proximity. In addition to cell cycle-related genes we have also identified, unexpectedly, genes beloging to other functional categories, e.g. transcription, stress and defence or signaling. We have determined the mRNA levels of genes belonging to different categories in cultured cells partially synchronized with aphidicolin. Most, but not all, of the genes identified *in silico* show a cell cycle-regulated expresion pattern.

7-12 Characterisation of root hair mutants of *Arabidopsis thaliana*

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The root epidermis of *Arabidopsis thaliana* is widely used as a model system to study the process of morphogenesis. There is a precise regulation of events that leads to the growth of a root hair and understanding the genetic control behind the growth of this cell type will provide a basis for studying other cell types in less accessible plant tissues. To gain a mechanistic understanding of root hair development we have commenced a screen to identify mutants, which affect normal development.
Feldmann T-DNA lines were screened for mutants that showed an altered root hair phenotype. Retesting of seed identified two true breeding mutants. Both mutants are in the Wassileewskija background and segregation analyses identified these mutants to be monogenic and recessive. The *flubber (flb*) mutant has abnormally short root hairs that are considerably fatter than the wild type. Further investigation using cryo-SEM showed branching along their length. Branches seem to occur opposite one another and are smaller than the hair itself. The *long branched root hair (lbrh)* mutant has root hairs that develop branching along their length. In some individual hairs, the branch was longer than the hair itself. The *lbrh* mutant is twice the length of the *flb* mutant although it is still shorter in length than the wild type. A defect in the elongation process may be responsible for the short phenotype observed in the *flb* mutant. The occurrence of branching may indicate a defect in polarity.

7-13 ANGUSTIFOLIA versus CtBP; What is the function of the AN protein in regulation of polar cell growth?

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In examining the mechanisms that govern the leaf width in *Arabidopsis*, we have found that the *AN* gene regulates polar cell elongation in the leaf-width direction (1,2). The *AN* gene encodes a member of the CtBP family, whose members act as transcriptional co-repressors in the animal kingdom. *AN* is the first member of the CtBP family isolated from plants (3). To determine whether the AN protein also functions as a co-repressor, we carried out a yeast two-hybrid analysis and a microarray analysis. The AN protein was found to self-associate, like other CtBPs. However, the domain required for self-association was localized to a region that differed from the domain in animal CtBPs. It was also found that the *an* mutant expresses some genes at a higher level than the wild type, suggesting that the *AN* gene might act as a repressor of such genes. The candidate target genes of the AN protein include *MERI5* which is thought to regulate loosening of the cell wall.
To understand the role of the polarity-dependent growth of each cell in determining the shape of the whole organ, we constructed transgenic *an* mutants expressing the *AN* gene under various promoters. The phenotypes of the resulting transgenics will be presented. Based on the results of gene analyses, the molecular mechanisms related to the regulation of leaf width will be discussed.
*This study was supported by the Monsanto Arabidopsis Microarray Program.
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7-14 Analysis of Cajal Body mutants in Arabidopsis

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Cajal Bodies (CBs) are small spherical structures located in the cell nucleus. They are found in both plant and animal cells, and are dynamic, mobile structures that frequently associate with the nucleolus. They contain small nuclear ribonucleoproteins (snRNPs), small nucleolar ribonucleoproteins (snoRNPs), nucleolar proteins, in addition coilin - a protein regarded as diagnostic for CBs. CBs were first identified by Ramon y Cajal a century ago but their precise function remains unknown, although it has been suggested that CBs may play a role in spliceosome assembly or may control the transport of splicing components within the nucleus.
We have developed an Arabidopsis line transformed with a U2B”::GFP construct, which enables the visualization of the CBs by fluorescence microscopy in living plants. Through EMS mutagenesis and screening of this line four distinguishable mutants have been identified; *no cajal body (ncb), small cajal body (scb), cap cajal body (ccb*) and *poly cajal body (pcb).* Both the *ncb* and *scb* mutants contain mutations within the gene coding for coilin. Interestingly, the plant version of coilin contains only a small region that is homologous to the forms found in other species, and does not include many of the important domains that have been identified, including the RG domain, which is important for interactions with SMN. Therefore, we are now interested in identifying proteins that interact with coilin and any domains that it may possess.

7-15 COBRA is part of a new family of putative GPI-anchored proteins

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Plant cell morphogenesis is largely determined by regulation of cell wall expansion. Point mutations in the *COBRA* gene result in a conditional cell expansion phenotype in root cells. This phenotype is dependent on high growth rate. The *COBRA* gene encodes a putative glycosylphosphatidylinositol (GPI) anchored protein likely to be localized to the plasma membrane-cell wall interface. Analysis of the *Arabidopsis* genome has revealed that *COBRA* is part of a multigene family consisting of twelve members, all predicted to encode putative GPI-anchored proteins. This family comprises two main classes, one of which is characterized by an extra N-terminal domain. Phylogenetic and expression analyses of these family members will be presented. In addition, we will describe potential loss of function mutant phenotypes in *COBRA* family members.

7-16 Studies of the Arabidopsis cochaperones the large FKBPs

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The co chaperones consist of several families of heat shock proteins and immunophilins which were found as associates and/or helpers of the classical chaperones hsp70 and hsp90. The FK506 binding proteins (FKBPs) were named immunophilins due to their property to bind the immunosuppresive drugs FK506 and rapamycin and are known also as peptidyl prolyl cis/trans isomerases (PPIases).Plants were shown to possess several families of PPIases namely the cyclophilins , FKBPs and parvulins.Our studies on the wheat FKBPs have shown that they possess chaperone activity , bind Hsp90, calmodulin and dynein.The large wheat FKBPs were able to exchange their mammalian counterpart the FKBP52 in binding via hsp90 to the steroid receptor complex and also to dynein. The overexpression in rice of the wheat truncated recombinant protein consisting only of the three PPIase domains (without the TPR and calmodulin domains)resulted in male sterile plants. Recently we have shown that over expression of wheat PPIase domains in Arabidopsis resulted in male sterile plants and plants with aberrated meristems.We have isolated knockout mutants of the Arabidopsis FKBP rof1 and have shown that the mutant is heat stress sensitive.In this study we show that the Arabidopsis large FKBPs ,the rof1 and rof2 are heat stress and developmentally regulated genes. At present we study the regulation of expression of the rof genes , search for their signal transduction pathway and putative target proteins. A working hypothesis about the target proteins of the rof genes will be presented.

7-17 The trichome branching gene, *FRC3*, encodes a novel, plant-specific calmodulin-binding protein

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We have been using the branching of trichomes on *Arabidopsis* as a model for how plant cell shape is controlled. A mutational approach to this process has identified at least 20 genes involved in the process of trichome branching. The *FRC3* gene has been shown to be an important positive regulator of trichome branching. Trichomes on *frc3* mutants have only two branches whereas wildtype trichomes have three or four branches. Analysis of the phenotypes of single and double mutants has shown that the function of *FRC3* is partially redundant to the function of *ZWI* which encodes a kinesin-like, calmodulin-binding protein (KCBP). To fully understand the role of *FRC3* in trichome branching, we cloned the *FRC3* gene using a map-based approach. The *frc3-1* allele, which was induced by fast neutrons, contains a deletion of 23,435 bp. Rescue of the *frc3* phenotype by one of the candidate genes covered by the deletion confirmed that we had identified the *FRC3* gene. *FRC3* encodes a novel protein that contains two IQ domains. These domains have been shown in other organisms to be involved in Ca2+-independent binding of calmodulin. The absence of any proteins with significant homology to FRC3 in the yeast, Drosophila, or human sequence databases suggests that this protein is plant-specific. Results from our analysis of wild-type and mutant plants overexpressing FRC3 will be presented, as well as analysis of the cytoskeleton in *frc3* mutants.

7-18 Mapping the activity spectrum of an ARF-GEF: GNOM and its role in the polar localisation of PIN1

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GNOM is required for alignment of cells with respect to each other and displays severe defects in the establishment of the embryonic axis. GNOM acts as a GDP/GTP exchange factor for G-proteins of the ARF class (ARF-GEFs). ARFs recruit COPI and clathrin coats to membranes and may thus regulate diverse trafficking events. Moreover, ARF-GEFs are the primary targets of Brefeldin A, which is widely used to elucidate trafficking processes. The pathway specificity of ARF-GEFs remains to be clarified, especially in plants. The Arabidopsis genome encodes for eight ARF-GEFs allowing for a certain degree of specificity of action. GNOM has been proposed to be involved in signal dependent trafficking events, leading to the coordinated polar localisation of auxin transport regulators such as PIN1 (1). BFA normally disrupts cycling of PIN1 and leads to its internalisation (2). Using a novel approach to address specificity of ARF-GEF action, we present direct proof that GNOM is regulating PIN1 transport. We introduced engineered BFA-resistant GNOM into gnom mutant backgrounds. This leads to BFA resistant PIN1 localisation. We investigate changes in BFA sensitivity of several markers in these lines in order to map the activity sites of the GNOM ARF-GEF. Moreover, we define the subcellular localisation of GNOM with respect to other subcellular markers.

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7-19 Cloning of a gene encoding a new member of glycosyltransferase family 8 of *Arabidopsis thaliana* by *Ds* element tagging

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Genomic sequence from Arabidopsis has revealed the presence of more than 359 glycosyltransferase orfs whose function are yet to be established (The AGI, 2000). These glycosyltransferases are classified in 52 families by bio informatics analysis; in which families 1, 2, 8 and 31 are those abundant ones (http://afmb.cnrs-mrs.fr/|pedro/CAZY). In the last few years, efforts have been made by many groups to characterise this huge family of genes which promise play important roles in plant development (Bowles et al., 2001, Keegstra and Raikhel, 2001). We report here the cloning of a new member of glycosyltransferase classified in family 8 by Ds tagging strategy. The Arabidopsis mutant, which was isolated in an F3 population following Ac transposase-mediated activation of a Ds element originally located on chromosome II. The disruption of the gene by Ds insertion caused severe defects in Arabidopsis including dwarf phenotype (therefore designated parvus) and failure of dehiscence that were partially rescued by high humidity condition, not by hormones or jasmonic acid. Sequences flanking the site of Ds insertion were isolated by IPCR and the amplified product was used to isolate both genomic and cDNA clones from the wild-type parvus locus. A comparison of sequences from both wild-type and mutant identified the site of insertion of Ds within the coding region of the parvus gene. Two revertant alleles were isolated, one of which contained a 7bp Ds excision footprint; a second contained Ds at a new location. The protein encoded by the parvus locus belongs to Glycosyltransferase family 8 which includes those involve in the synthesis of bacterial lipopolysaccharide, glycogen, and galactinol. Sequencing and analysis using bio informatics tools reveal also a large number of EST clones widely present in other plant species, including monocot and dicots. Cellular fractionation method has been employed to show that PARVUS localises in ER and Golgi, the home place for Glycosyltransferases that involve in the synthesis of the complex polysaccharide of cell wall.

7-20 Trichome-specific expression in Arabidopsis is regulated by intronic enhancer elements

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The *Arabidopsis* trichome structures posses a very active cysteine and glutathione biosynthesis rate. The function of these unicellular trichomes is related with defense against insect attack and with detoxification processes. Analysis by *in situ* hibridization demonstrated that the *Atcys-3A* gene, coding for the cytosolic isoform of the O-acetylserine(thiol)lyase enzyme, is highly expressed in this cell type as well as other genes involved in cysteine and glutathione biosynthesis. We are characterizing the promoter region of the *Atcys-3A* gene by fusion to a GFP reporter gene. A 1809 nt fragment containing the promoter, the 5´untranslated region, the first intron and two exon fragments is able to mimic the expression pattern of the *Atcys-3A* gene when fused to GFP. This expression pattern include a high level of GFP accumulation in trichome cells and a constitutive basal level in root and leaf tissues. Deletions of the full length promoter from the 3´-end demonstrate that the first intron contained in the 5´-untraslated region is necessary to drive the reporter expression within trichomes. This result suggest the presence of cis elements acting as enhancer for trichome-specific tissue expression.
Functional characterization of the 1809 nt DNA fragment in other species such as tobacco and peppermint fused to GUS and GFP reporter genes indicate that this fragment is also able to confer trichome expression in glandular trichomes. Although the expression driven by the full-length promoter in not unique in glandular trichomes, this result may have important biotechnological applications.

7-21 Isolation and characterisation of Aurora/Ipl1-like kinases in *Arabidopsis thaliana*

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Members of the Aurora/Ipl1 family of serine/threonine kinases are key regulators of chromosome segregation and cytokinesis. These kinases have been isolated and characterized for yeast, Drosophila, C. elegans and a number of vertebrates. In this species the function of these kinases is closely linked to microtubule dynamics, chromosome segregation and other regulatory processes. It has been demonstrated that Aurora-kinases are capable of histone H3 phosphorylation. Phosphorylation of the core histone H3 at serine 10 has been linked to the chromosome condensation/segregation process in a number of species. The function of the phosphorylation of histone H3 in plants is uncertain. To study the relationship between H3 phosphorylation and chromosome condensation/segregation mitotic Arabidopsis cells were immunostained using an antibody that recognizes phosphorylated histone H3 at serine 10. At mitosis histone H3 phosphorylation starts at prophase and ends at telophase and compared with other chromosome regions the pericentromeric chromatin is H3 hyperphosphorylated.
Based on sequence comparison we have identified and cloned three putative Aurora-kinases orthologues in Arabidopsis. A strong expression of all Aurora-kinases was detected in mitotically and meiotically active tissues. To analyse whether the modulation of kinase activity influences the phosphorylation status of histone H3 and segregation behaviour of chromosomes, overexpression/inactivation studies of Aurora-kinases will be performed.

7-22 CDKa;1 interacting kinesin motor proteins implicated in cell division

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The cyclin dependent kinase ArathCDKa;1 interacts in two hybrid tests with two Arabidopsis kinesin motor proteins. EGFP-chimeric proteins containing the full kinesin protein or peptide fragments were expressed in BY-2 suspension cells and Arabidopsis. In contrast to the EGFP protein, the kinesins N-terminally fused to EGFP were excluded from the nucleus in interphase cells. Fluorescence was observed throughout the cytoplasm, not being associated with the microtubular network. In mitotic cells the spindle was diffusely labeled similar to what we could observe for cells that expressed the free EGFP protein. However, the moment a phragmoplast emerged, bright fluorescence appeared at the cell center labeling the midline of the young phragmoplast. At a later stage, the fluorescence expanded along with the growing phragmoplast and developed into a ring-like structure. The fluorescence was strong at the midline suggesting that the EGFP-kinesin proteins were associated with a sub-region of the phragmoplast corresponding to the position where the positive ends of the microtubules intersect. The C-terminal half of the protein contained all the necessary information for targeting to the phragmoplast midline. The motor domain is N-terminal located outside of the C-terminal moiety. Perhaps a yet unrecognized microtubule binding site is present in the C-terminal part of the kinesins, or alternatively, indirect protein interactions are mediating phragmoplast localization. The specific association with the phragmoplast midline is in accordance with an interaction with CDKA;1 which was also shown to bind the midline in cytokinetic cells. Phenotypic analysis of Arabidopsis plants expressing kinesin fragments fused to EGFP further support a role in cell division.

7-23 Functional analysis of the Gg subunits of heterotrimeric G proteins in plants

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Heterotrimeric G proteins are involved in various signaling pathways. Whereas many different G protein subunits are described in mammals only one gene encoding a Ga and a Gb subunit is found in the genome of *Arabidopsis thaliana*, respectively. In contrast, two genes encoding Gg subunits (AGG1, AGG2) have been identified recently in Arabidopsis (Mason and Botella, 2000; 2001). In this light it is very interesting whether the two Arabidopsis Gg subunits may have different functions and/or localisations, in which way they participate in signaling pathways, and how heterotrimeric G proteins in plants redeem their low diversity.
By transient expression assays of GFP fusion proteins we obtained evidence for in planta interaction of AGG1 with Gb and for different membrane localisations of AGG1 and AGG2. When expressed alone, GFP-AGG1 is located to membranes in about 50% of the cells analysed whereas clear membrane localisation only occurs when co-expressed with Gb. However, GFP-AGG1 is more associated with endomembranes than with the plasma membrane. By contrast, even without co-expression of Gb, GFP-AGG2 shows a very prominent localisation at the plasma membrane. We also want to characterise the interaction of the subunits of heterotrimeric G proteins biochemically and identify partners of the Gbg dimers. To this end, we raised antibodies against all G protein subunits from Arabidopsis and generated transgenic Arabidopsis lines that express AGG1 fused with a novel protein tag. In addition, we are using a three hybrid approach to identify proteins that interact with the Gbg dimers.

7-24 Plasma membrane localized Calcium-Dependent Protein Kinases (CDPKs) in Arabidopsis

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In plants, calcium-dependent protein kinases (CDPKs) are the predominant calcium-stimulated kinases and are known to be involved in many cellular processes. However, the details of how specific CDPKs function in plant cells are not well understood. CDPK enzymatic activity has previously been detected in many locations in plant cells, including the membrane fraction. Computer predictions indicate that CDPKs do not have transmembrane domains. Therefore, CDPKs must use other mechanisms for membrane localization. We have observed that 29 of the 34 CDPKs contain potential myristoylation motifs at their amino termini. Myristate is a 14-carbon fatty acid that is attached co-translationally to the amino-terminal glycine of a nascent protein. Myristoylation can facilitate membrane binding and /or protein-protein interactions. Arabidopsis CDPK isoform, AtCPK5, has been demonstrated to be myristoylated in a cell-free wheat germ extract and myristoylation was prevented by converting the glycine at the proposed site of myristate attachment to alanine (G2A). We are studying both the subcellular membrane location of AtCPK5, as well as the role that myristoylation plays in membrane association. Subcellular localization studies were conducted using both aqueous two-phase system and sucrose density gradient fractionation of plant microsomes. AtCPK5 appears to be associated with the plasma membrane and the G2A mutation abolishes AtCPK5 membrane association in plants. A recombinant protein, consisting of the first sixteen amino acids of AtCPK5 at the amino-terminus of soluble beta-glucuronidase, was also targeted to the plasma membrane indicating that the amino terminus of the AtCPK5 protein contains the necessary information for plasma membrane localization.

7-25 Rab GTPases regulating the endocytic pathway in *Arabidopsis thaliana*

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Rab/Ypt GTPase regulates the fusion events of vesicles and target membranes through its GTPase cycle. It is well known that many Rab GTPases play important roles in the regulation of mammalian endocytic pathway, however, molecular mechanisms of the endocytosis in plant cells are largely unknown. The *Arabidopsis* genome contains at least two, three and seven homologs for Rab5, Rab18 and Rab7, respectively. *Arabidopsis* also has a unique type of Rab/Ypt GTPase, Ara6, which shows the high similarity to the Rab5 group. We isolated these putative endocytic Rab GTPases and examined their subcellular localization and function. Ara6 and Rab5 orthologs (Ara7 and Rha1) are localized on the punctate organelle, which turned out to be endosomes. On the other hand, Rab7 homologs (AtRab71 – AtRab77) are mainly localized on the vacuolar membrane, and Rab18 homologs are on the vacuole and dots in the cytosol. To observe the dynamics of endosomes in living cells, we constructed a suspension cultured cell expressing GFP-tagged Ara6. In this meeting, we report a dynamic cytoskeleton-dependent movement of endosomes as well as the unique localizations of several Rab proteins in the endocytic pathway.

7-26 The endothelium-specific expression of the *BANYULS* gene is transcriptionally regulated during seed coat development

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The *BANYULS (BAN*) gene of Arabidopsis encodes an enzyme of the flavonoid biosynthetic pathway (putative leucoanthocyanidin reductase), required for the formation of proanthocyanidins (PAs), specifically in the endothelial cell layer of the seed inner integument. We have visualized the spatio-temporal expression of the GUS and GFP reporter genes driven by the *BAN* gene promoter in transgenic Arabidopsis plants. Reporter gene products were detected only in the endothelium of ovule and developing seed. They appeared first at the level of the micropylar and chalazal poles in mature ovules. After fertilization, their accumulation extended further in the remaining part of the endothelium (body), picking at around 3 days after fertilization. It is concluded from these observations that the endothelium is organized into three domains exhibiting distinct developmental time courses of *BAN* expression. Altogether, our data suggest that *BAN* promoter activity in developing seeds coincides with the pattern of *BAN* gene expression previously defined by RT-PCR analysis or in situ hybridization, and correlates with the presence of PAs detected by the vanillin histochemical assay. The activity of the *BAN* promoter was also monitored by fusing it to the cytotoxic *BARNASE* gene. Seeds with an ablated endothelium layer were obtained on transgenic plants having no additional defect. This experiment confirms the endothelium-specific activity of the *BAN* promoter. It also provides us with an invaluable material to study the role played by the endothelium layer in Arabidopsis seed ontogeny, and particularly cell-cell interaction mechanisms that take place during integument development.

7-27 The Arabidopsis WEE1 kinase controls cell cycle progression by modification of the phosphorylation status of the CDKs

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The essential processes of the cell cycle are controlled by the ordered action of a protein complex composed of a catalytic subunit named Cyclin-Dependent Kinase (CDK) and positive regulatory elements named cyclins. The CDK/cyclin complex activity is mainly regulated by modification of the phosphorylation status of the kinase. In yeast and mammals the CDK/Cyclin complexes formed during S and G2 phases are maintained in an inactive form through inhibitory phosphorylation by a family of kinases called WEE1. The prompt activation of the CDK/cyclin activity at the G2/M transition is controlled by dephosphorylation.
In the Arabidopsis genome a single wee1 gene has been detected. We have isolated the corresponding cDNA named Arath;wee1 and confirmed its functional homology with the wee1 family in a yeast two-hybrid assay and by overexpressing it in the fission yeast. The purified protein was able to phosphorylate active purified complexes inhibiting CDK activity. In Arabidopsis cell suspension synchronized by aphidicoline, wee1 was expressed, in response to an activation of the DNA replication checkpoint, maintaining the CDKA in an inactive phosphorylated form. The analysis of a GFP-WEE1 fusion protein shows preferential WEE1 localisation in the interphase nucleus and its removal during mitosis confirming the role of WEE1 in control of CDK/Cyclin complex activity. As the overexpression of WEE1 is lethal in BY-2 cells we chose to use an inducible system to overexpress it under the control of different promoters in BY-2 cells and in Arabidopsis. Data concerning the analysis of the transgenic cells and plant lines will be presented.

7-28 Functional analysis of *SPIRAL1-LIKE* genes

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The *Arabidopsis SPIRAL1 (SPR1*) gene encodes a small polypeptide with yet unknown biochemical functions. Mutation in *SPR1* causes right-handed twisting in the epidermis of roots and etiolated hypocotyls presumably due to unsynchronized elongation of the epidermis and inner cortex, as well as increase in the number of trichome branching. Consistent with its effect on cell elongation process, cortical microtubules of the *spr1* root epidermis are oriented obliquely relative to the root long axis. SPR1::GFP fusion protein was found in a fiber-like structure on the cell surface suggesting its association with cortical microtubule arrays. *Arabidopsis* genome contains five *SPR1* homologs designated *SPIRAL1-LIKE (SP1L*) genes, *SP1L1* through *SP1L5*. SPR1 and SP1Ls share high sequence identity in both N- and C-terminal regions. Expression analysis by RT-PCR indicated that *SPR1* and *SP1Ls* are expressed in distinct organ-specific patterns, except for *SP1L1* that showed no detectable level of transcripts in all organs tested. Over-expression of *SP1L2* through *SP1L5* by the CaMV35S promoter rescued the twisting phenotype of *spr1* mutants, indicating similar biochemical functions between SPR1 and SP1Ls. A few transgenic lines, however, enhanced the twisting and trichome branching phenotype of *spr1*. These results indicate that while SPR1 and SP1L proteins share at least partially overlapping functions in modulating microtubule functions, different members may participate in different cell elongation events depending on the cell types.

7-29 Cell dynamics at the shoot apical meristem

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The aerial parts of the plant are generated by groups of rapidly dividing cells called shoot apical meristems (SAMs). SAMs are highly stable and organised structures, which are divided into functionally distinct domains. Recently, a number of factors involved in meristem function have been identified, such as transcription factors defining cell identity in the different domains and elements of signalling cascades. How these 'upstream' regulators influence and integrate cell behaviour is still an open question. To study this aspect we have developed a technique to visualize living meristems and cells This method, which combines GFP marker lines and vital stains allows us to follow cell proliferation, cell expansion and cell differentiation in individual meristems for several days. In a first series of experiments, using primordium promoters (ANT and LFY) driving GFP expression, we followed the recruitment of meristematic cells in the incipient flower primordia. This suggested that cells preferentially activated the reporter genes just after cell division, i.e. while they are in early G1. To investigate this further, we treated meristems with mitosis inhibiting drugs. Interestingly, when cell division was blocked using Oryzalin, (a microtubule depolymerising drug) cell expansion and cell differentiation proceeded normally. In addition, cells at the periphery of the meristem and in the young primordia expanded faster than those at the meristem centre. This showed that differential cell expansion rates and cell differentiation do not depend on the cell cycle. Implications of these and other experiments will be discussed.

7-30 The *ASB1* and *ASB3* genes encode novel, plant-specific proteins involved in trichome branch initiation

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To understand the how trichome branching is controlled, we have screened for mutations that affect trichome branch initiation. We isolated a new group of mutants that show large effects on trichome branch position and length, but little effect on trichome branch number. We named this group *asymmetric branch (asb*) mutants. This group contains at least 4 loci (named *ASB1, ASB2, ASB3*, and *ASB4*). Double mutants of *asb* with certain *zwi* alleles have novel trichome phenotypes. This suggests that the ASB proteins interact with ZWI. *ZWI* encodes a kinesin-like motor molecule (KCBP) and provides a natural link to the involvement of the ASB proteins in the control of microtubule organization or function. To further understand the role of the *ASB* genes, we used a map-based approach to isolate them. Here we report that *ASB1* and *ASB3* encode novel, plant-specific proteins. Progress toward intracellular localization of these proteins and the affects of the *asb* mutations on the cytoskeleton will be presented.

7-31 *MUM4* and *PRAIRIE* regulate mucilage accumulation and cytoplasmic rearrangement during seed coat differentiation in Arabidopsis

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Fertilization of an ovule leads to the activation of seed coat differentiation as well as the development of the embryo and endosperm. The seed coat consists of multiple specialized cell layers, including, in some species, the presence of polysaccharide mucilage in the epidermal layer. In *Arabidopsis*, analysis of the differentiation of the mucilaginous epidermal cells has shown that this is a complex process including the biosynthesis and secretion of a large quantity of pectin (mucilage), cytoplasmic rearrangement, and secondary cell wall biosynthesis. Several genes that affect the amount of mucilage and the degree of cytoplasmic rearrangement have been described, including *MUCILAGE MODIFIED 4 (MUM4), TRANSPARENT TESTA GLABRA 1 (TTG1), GLABRA 2 (GL2)* and *MYB61*. While *GL2* and *TTG1* affect epidermal cells throughout the plant, mutations at *MUM4* and *MYB61* only appear to affect the seed coat. *GL2* and *TTG1* encode a putative homeodomain transcription factor and a WD40 repeat containing protein, respectively, and are thought to be global regulators of epidermal development. Conversely, *MYB61* encodes an R2R3-MYB protein localized to the seed coat and vascular tissue. In order to learn more about the control of seed coat differentiation, the cloning of *MUM4* has been undertaken. In addition, we have identified a novel gene that also affects mucilage cell differentiation: *PRAIRIE (PRA). pra* mutants have a similar phenotype to *mum4* mutants: a reduced amount of mucilage and flattened secondary cell wall. The characterization of *pra* will be described.

7-32 ASK1-dependent disassociation of two non-sister chromatids of the bivalent in Arabidopsis

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The *ask1-1* mutant, which is mutated in the *Arabidopsis* Skp1 homolog ASK1, exhibits non-disjunction of homologous chromosomes during male meiosis. Presumably, a structure for adhering the homologous chromosomes is stabilized in *ask1-1*. To gain insight about the nature of such adhesion, we examined dynamics of chromosomal morphology and the transcription of *ASK1* and its closest homolog *ASK2* during the different stages of male meiosis in the wild-type (WT) and *ask1-1* plants. We found that during late diplotene, two homologous chromosomes of each bivalent were hardly distinguishable in *ask1-1*, but they were clearly distinguishable in the WT. This observation might be interpreted that the mutant bivalents are held together at sites much closer to the centromeres than those in the WT. During meiosis II in *ask1-1*, some chromosomes separated from the bivalents and others remained associated. On average, there were about 15 DAPI-stained chromosome units per microspore mother cell in *ask1-1*. Some of these chromosome units appeared to be comprised of subunits. These results are consistent with the assumption that 10 single chromosomes and 5 chromosome duplexes are present in *ask1-1*. We also found that *ASK1*, but not *ASK2*, was transcribed during prophase I, and that both were transcribed from metaphase I to the tetrad stage, suggesting that the ASK1 protein is non-redundant only during prophase I. Based on these and other results, we propose that two non-sister chromatids within each bivalent normally associate during pachytene and/or diplotene, and they disassociate only during diplotene via an SCF ubiquitin ligase-regulated process.

7-33 Subcellular dynamics in the early stages of the compatible interaction between *Arabidopsis thaliana* and the powdery mildew *Erysiphe cichoracearum*

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Improved live cell imaging techniques using green fluorescent protein (GFP) and confocal microscopy provide powerful tools for studying early stages of plant-microbe interactions. Eight different plasma membrane (PM) marker lines tagged with GFP were infected by *Erysiphe cichoracearum* and observed under a confocal microscope to study the characteristics of the extrahaustorial membrane (EHM). This study showed that GFP signal of all eight PM marker lines were excluded from the EHM and only remained around the haustorial neck area in the stage 4 (16 to 24 hours after infection). It supported the idea that the EHM is a unique membrane that is different from the plasma membrane of *Arabidopsis* epidermal cells. The two possibilities of rapid differentiation of the plasma membrane or de novo synthesis of the EHM during the young haustorium development were considered. To test these hypotheses, we observed the dynamics of organelles in infected cells especially around the penetration peg at early time points. Various double marker lines expressing both cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) were generated and used to visualize the movements of organelles in response to the pathogen infection, in presence or absence of latrunculin B (LatB).

7-34 Functional analysis of the cellulose synthase genes *CesA1*, *CesA2* and *CesA3*

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Polysaccharide analyses of mutants link several of the glycosytransferases encoded by the ten *CesA* genes to cellulose synthesis. We transformed with antisense constructs to investigate the functions of *CesA2 (AthA)* and *CesA3 (AthB*), genes for which cellulose-deficient mutants are not yet available. Plants expressing antisense *CesA1 (RSW1)* provided a comparison with a gene whose mutant phenotype points mainly to a primary wall role. The antisense phenotypes of *CesA1* and *CesA3* were closely similar and correlated with reduced expression of the target gene. Reductions in cell length rather than cell number underlay the shorter bolts and stamen filaments. Surprisingly seedling roots were unaffected in both *CesA1* and *CesA3* antisense plants. In keeping with the mild phenotype compared to Rsw1-, reductions in total cellulose levels in antisense *CesA1* and *CesA3* plants were at the borderline of significance. We conclude that CesA3 like CesA1 is required for deposition of primary wall cellulose. To test whether there were important functional differences between the two, we overexpressed *CesA3* in *rsw1* but were unable to complement that mutant's defect in CesA1. The function of CesA2 was less obvious but, consistent with a role in primary wall deposition, the rate of stem elongation was reduced in antisense plants growing rapidly at 31ºC. A promoter-GFP fusion showed expression in the root meristem but with expression rapidly becoming confined to vascular tissue in older parts of the root and in other organs. CesA2 may therefore also contribute to secondary walls but no experimental evidence yet exists for this function.

7-35 Cellulose microfibril alignment and cellulose synthesis in the microtubule-defective mutant *mor1*

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In plants the direction of cell elongation and thus cell shape is determined by several factors, including both cortical microtubule and cellulose microfibril organization. Disturbing cortical microtubules or cellulose microfibrils causes the cells to expand isodiametrically. Isotropic cell expansion can e.g. be observed in the temperature-sensitive microtubule organization mutant (*mor1*) from Arabidopsis. At the permissive temperature (21ºC) cortical microtubules are well-ordered and transverse to the axis of cell elongation as in wild type. But at the restrictive temperature (31ºC) cortical microtubules are shorter and disorganized, and cells swell radially. Cortical microtubules are generally thought to control the direction of cellulose deposition by guiding the cellulose synthase enzyme complexes in the plasma membrane. In this context we checked cellulose microfibril alignment in the *mor1* mutant at the restrictive temperature (31°C). We first inhibited cellulose biosynthesis with the herbicide DCB and then observed the recovery from DCB treatment at the restrictive temperature (cortical microtubules disorganized). Our experiment demonstrates that cellulose microfibrils can be deposited in parallel order even though cortical microtubule patterns are greatly disrupted.
But what is altered in *mor1* cell walls to account for the loss of growth anisotropy? Analysing the carbohydrate composition of cell wall extracts, we show that *mor1* seedlings grown at 31°C produce similar amounts of cellulose as wild type. The contents of non-cellulosic sugars are also not significantly changed, except that in WT at 31ºC the non-cellulose fraction is very high in glucose, whereas this cannot be observed in *mor1*. This finding may be a key for the radial swelling phenotype of *mor1*.

7-36 Posttranslational quality control of mutant MLO proteins

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Barley *Mlo* encodes a membrane protein with seven transmembrane domains. Lack of the wild type protein leads to broad spectrum disease resistance against the pathogenic powdery mildew fungus *Blumeria graminis* f. sp. *hordei*. Certain single amino acid substitutions in the MLO protein have been previously shown to reduce or even eliminate detectable MLO protein levels in barley membrane fractions. Further it has been shown *in vitro* for some representative mutant alleles that translation as well as membrane insertion occurs as in the wild type. These observations led to the conclusion that a posttranslational quality control mechanism might be responsible for the selective degradation of proteins encoded by mutant *Mlo*-Alleles.
Translational fusions of wild type and mutant *Mlo* alleles to different reporter genes and their transient and stable expression in *Arabidopsis thaliana* and *Saccharomyces cerevisiae* were chosen as experimental systems for elucidating mechanisms underlying the observed instability of mutant MLO proteins. Application of a Dual Luciferase System allows the quantification of relative protein stability of mutant MLO proteins in comparison to the wild type protein. First results indicate a strong conservation of MLO quality control between monocot and dicot plant species and yeast. Experiments in certain yeast mutants strongly indicate the participation of the proteasome in the degradation of unstable MLO proteins. We therefore propose that a process similar to the proteasome-dependent endoplasmic reticulum associated protein degradation (ERAD) might be responsible for the quality control of mutant MLO proteins.

7-37 SGR3/AtVAM3, t-SNARE, functions in shoot gravitropism in endodermal cells.

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Plant shoots and roots show negative and positive gravitropism, respectively. We have isolated a number of *shoot gravitropism* (*sgr*) mutants of Arabidopsis to elucidate the molecular mechanism of gravitropism. *sgr3-1* mutant, which is recessive, exhibited reduced gravitropic response in the inflorescence stems and their lateral shoots grew horizontally. It have been shown that the endodermal cells are the gravity-sensing cells of inflorescence stems, which contain sedimented amyloplasts. However, some amyloplasts were localized to upper side of the endodermal cells in *sgr3-1* mutant.
SGR3 encodes a syntaxin, AtVAM3, which have been cloned as a homologue of yeast Vam3p. It has been suggested that AtVAM3 is localized to prevacuoler compartment (PVC) and vacuole and functions in post-Golgi vesicle trafficking. sgr3-1 had a single base mutation causing a single amino acid change adjacent to a SNARE motif. Interestingly, we have also cloned other SNARE, ZIGZAG/AtVTI11, of which mutation cause abnormal gravitropism and the amyloplasts in the endodermal cells located highly similarly to those of sgr3-1. Endodermis-specific expression of SGR3 and ZIG using SCR promoter could complement the abnormal shoot gravitropism of each mutants. Protein-protein interaction between SGR3/AtVAM3 and ZIG/AtVTI11 was detected immunologically in endodermal cells. sgr3-1 mutation reduced the affinity between AtVAM3 and AtVTI11(or other SNAREs). These results suggest that vesicle trafficking from trans-Golgi network to PVC/vacuole in the endodermal cells by specific SNARE complex contaning SGR3 and ZIG play important roles in shoot gravitropism.

7-38 Phosphorylatory regulation of a dual-affinity nitrate transporter, CHL1, in Arabidopsis

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For most of plants, nitrate is their primary nitrogen source. The concentration of nitrate in the soil could vary several orders of difference. To maximize the uptake efficiency, plant evolved two uptake systems, one high-affinity and one low-affinity. These two systems were traditionally thought to be genetically distinct, and indeed, two families of nitrate transporter genes, NRT1 and NRT2 have been identified in Arabidopsis and thought to be responsible for low- and high-affinity nitrate uptake, respectively. However, recently, Arabidopsis nitrate transporters, CHL1, were shown to have dual-affinities. This is a relatively new concept, and nothing is known about how a dual-affinity transporter works and the advantages of having a dual-affinity transporter. In this study, we demonstrate that phosphorylation plays a major role for the switch of CHL1 between high- and low-affinity modes: when phosphorylated at threonine residue 101, CHL1 functions as a high-affinity nitrate transporter, whereas, when dephosphorylated, it functions as a low-affinity nitrate transporter. Our evidences include (1) nitrate uptake studies of CHL1-injected Xenopus oocytes treated with protein kinase activator or inhibitor, (2) kinetic analysis of Xenopus oocytes injected with site-directed mutagenized CHL1 cRNA, (3) in vitro CHL1 protein phosphorylation study and (4) plant complementation of low-affinity defect but not high-affinity defect of chl1 deletion mutant by a CHL1 cDNA with the putative phosphorylation site T101 eliminated. This regulatory mechanism could serve as a fast adaptation for plant to adjust their low- and high- uptake capacities in response to environmental changes and compete for limited nitrogen.

7-39 The Arabidopsis dynamin-like GTPase protein, ACW2/ADL1Ap, is required for cell differentiation, organizing cortical microtubules and cellulose biosynthesis

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Plant cell wall biosynthesis is thought to be closely related to cell division, differentiation and development. However, the molecular mechanisms of cell wall biosynthesis are poorly understood. To analyze the mechanisms, we have studied an *Arabidopsis* temperature-sensitive *acw2 (altered cell wall 2)* mutant that shows the deficiency of cell elongation and cellulose biosynthesis. Our characterization of *acw2* mutant revealed that ACW2 has pleiotropic effects on cytokinesis, tissue (cell) differentiation, and organizing cortical microtubules as well as cellulose biosynthesis in the plant. The mutant had a lot of cell wall stubs that are very similar to those observed upon caffeine inhibition of cytokinesis in plants. The radial pattern in *acw2* root meristem was altered distinctly under non-permissive temperature. Expression of *CPC* gene that is a marker of root hair development was also suppressed in the roots. In addition, confocal laser scanning microscopy revealed disorganization of cortical microtubule array in the petiole cells. We cloned the *ACW2* gene by map-based cloning. The gene encodes the dynamin-like large GTPase protein ADL1Ap which has been reported as a cell plate-associated protein. These results show that the dynamin-like protein ACW2/ADL1Ap is required for cellulose biosynthesis, tissue (cell) differentiation, and organizing cortical microtubules as well as cell plate formation.

7-40 Expression of the CDK inhibitor ICK1/KRP1 in Arabidopsis trichomes reduces endoreduplication levels and cell size and leads to cell death

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A correlation between cell size and DNA content has been observed in many cell types. This correlation is very obvious in endoreduplicating *Arabidopsis* leaf hairs (trichomes). In contrast, studies on a dominant negative CDK version and, recently, on cell cycle inhibitor proteins have shown that cell size can be uncoupled from cell cycle progression and DNA content. To address this apparent discrepancy we expressed *ICK1 / KRP1* in trichomes and analyzed the function of the CDK inhibitor in this cellular context. We observed that *ICK1 / KRP1* expression reduced the endoreduplication levels and, accompanying, the cell size; a reduction in cell size could also be obtained by directly inhibiting DNA replication by aphidicolin treatment. Our data emphasizes the dependency of cell growth on the DNA amount. However, we could detect a high degree of variation in the cell size to nuclear size ratio indicating that cells can grow within a certain range without an increase in DNA content. We further show that the inhibitory function of ICK1/KRP1 *in planta* is specific to a C-terminal domain and is negatively controlled by a N-terminal domain. Moreover, we found that *KRP* expression also interfered with cell survival, linking cell cycle progression, differentiation and cell death in plants. The possible targets of ICK1/KRP1 function in the trichome cell will be discussed.

7-41 Ectopic D-type cyclin expression induces not only DNA replication but also cell division in Arabidopsis trichomes

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Although the mechanisms controlling the two cell cycle checkpoints G1-S and G2-M are well studied, it remains elusive how they are linked in higher eukaryotes. In animals, D-type cyclins have been implicated in the control of cell cycle progression in mitotic as well as in endoreduplicating cells. We use *Arabidopsis* trichomes as a model cell to test the function of two D-type cyclins on cell growth and development in plants. In contrast to what is found in animals, we show that the expression of the D-type cyclin *CYCD3;1* in endoreduplicating *Arabidopsis* trichome cells not only induced DNA replication but also cell divisions. The possibility of a plant specific action of D-type cyclins at the entry into mitosis will be discussed.

7-42 Roles of Ca^{2+}-pectate-binding peroxidases in the control of the cell wall processes

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In higher plants, peroxidases exist as multigenic family. We have shown that an anionic peroxidase from zucchini (APRX) can bind to the Ca2+-pectate complex owing to a domain composed of clustered arginines. No specific function has been attributed to this APRX but few results and observations show that APRX might have a function in the lignin synthesis or control of elongation with the auxin catabolism.

To understand the function of this APRX and the role of its binding activity, we need to determine (i) its cellular localization using GFP fusion protein, (ii) the specific activity of APRX (iii) the phenotype of transgenic *Arabidopsis* plant carrying different cDNA constructs (APRX, APRX with no binding activity) and (iv) the *Arabidopsis* homologous.

Furthermore, a group of five *Arabidopsis* peroxidase containing a similar domain to the Ca2+-pectate binding motif BXXXXXBXXB of APRX (where B is a basic amino acid) can bind to the complex Ca2+-pectate. To investigate *in vivo* the role of these peroxidases, we are looking for T-DNA insertion mutants and generating double-stranded RNA silencing mutants with the purpose of reducing or suppressing the expression of all the peroxidases. We have also studied the specific cellular localization with the GFP fusion protein.

We expect from the present work to show that the binding of proteins to pectins is an essential process for the localization and the control of the enzymatic reactions occurring in cell walls as lignification, cell elongation or polymers cross-linking.

7-43 *TBT* gene is required for root patterning in *Arabidopsis thaliana*

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The Arabidopsis primary root has simple radial organisation set down during embryogenesis and thought to be maintained by positional cues. Different cell types are organised in concentric rings surrounding the stele tissue. The root epidermis is further specified into two cell types – hair forming cells and non-hair forming cells, organised in specific cell files. The differentiation of these two cell types depends on their location relative to the underlying cortical cell walls, indicating a role for cell wall localised positional information. The Arabidopsis root hair has been used as a model system because of the relatively easy identification of mutants and the molecular characterisation of the genes.
tablut (tbt), an EMS induced mutant, has defective epidermal sub-specification; ectopic root hairs form in non-hair cell positions, ectopic non-hairs form in hair cell positions. Morphological and histological characterisation of mutant plants has been performed and indicate that the gene defined by this dominant mutation may be involved in cell wall or cytoskeleton formation. The expression of non-hair cell specific molecular markers is altered in the meristem as well as in the elongation and differentiation zones. The misaligned cell walls and cell divisions and the defective root patterning observed in this mutant suggest that the TBT gene is active in early stages of root development. Genetic characterisation and mapping of the gene have started in order to confirm this hypothesis.

7-44 Initial step of root-hair morphogenesis regulated by the transcription factor GLABRA2

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Arabidopsis homeobox gene, *GLABRA2(GL2*) , encodes a HD-ZIP-START transcription factor which has been thought to regulate development of epidermal cells in many cases. To investigate the role of GL2 in root-hair cell differentiation, we determined a target gene of GL2 using a reverse genetical approach. Expression of a modified GL2 protein (GL2dAD-VP16), in which the N-terminal region was replaced by the transactivating domain of VP16, caused ectopic root hairs or root-hair-like organs. Using transgenic plants expressing GL2dAD-VP16 glucocorticoid-inducibly, we identified a *PLD* gene as a target gene of GL2. An in vitro binding experiment revealed a specific interaction between the DNA-binding domain of GL2 and a promoter region of the *PLD* gene. The gene is expressed in root-hair cell files, where *GL2*is not expressed. On the other hand, in *gl2* mutant plants, it is expressed in all the cell files of root epidermis. From these results, we concluded that GL2 makes the cell-file-specific root-hair pattern by repressing the *PLD*gene in non-root-hair cell files. Phospholipid signals are thought to control animal cell morphogenesis through the regulation of cytoskeletal rearrangement and vesicle trafficking. Our results suggest that an initial event in cell morphogenesis involves phospholipid signaling also in plants.

7-45 An important role of a ribosomal releasing factor 1 (RF1) in chloroplast development based on the analyses of a *Ds*-tagged albino 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 the *Ac/Ds* transposon tagging system. One of the albino mutants, designated *apg3* (for albino and pale green mutant 3), could not survive beyond the seedling stage when germinated on soil. A gene encoding a ribosome releasing factor 1 (RF1) homologue was disrupted by an insertion of *Ds* transposon in *apg3*. The *APG3* encode a protein with a transit peptide that functions in targeting the protein into chloroplasts. Since the chloroplast of *apg3* plants contains few internal thylakoid membrane, *APG3* is involved chloroplast development.

The amounts of chloroplast proteins related to photosynthesis were examined in the *apg3* mutant by immunoblot analysis. Analyzed proteins were thylakoid proteins D1, LHC and OE23, and soluble proteins Rubisco LSU and SSU. The chloroplast proteins were no detected in *apg3* mutant. By contrast, transcription of both nuclear and chloroplast genes for chloroplast proteins was normal even in *apg3* mutant. This indicates an important role of the APG1 protein, a RF1 homologue, in correct translation of chloroplast proteins and uncoupling of transcription and translation in chloroplast protein biosynthesis. The decrease of nuclear-encoded chloroplast proteins in *apg3* may be a secondary event caused by the significant decrease of internal thylakoid membranes. Moreover, an *E. Coli* RF1 mutant was complemented with APG3 encoding chloroplast RF1 (cpRF1).

These results indicate that *APG3* gene plays an important role in the translation machinery of of the chloroplast.

7-46 The use of the Chimeric Repressor Interference System (CHRIS) to analyse the function of plant transcription factors

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Many types of plant response signals such as environmental responses and physiological feedback are integrated at the level of cell-specific transcription, making transcriptional control probably the most important level of gene regulation in eukaryotes. It has been estimated that transcription factors comprise c.6% of the total number of genes in *Arabidopsis*. However, only a small percentage has been functionally characterised by mutational analysis. In order to better understand plant regulatory networks, we have developed a new tool (CHRIS), to convert plant transcription factors into dominant-negative repressors *in planta*. In the ***C****himeric* ***R****epressor* ***I****nterference* ***S****ystem*, specific transcription factors are fused to the *ENGRAILED* repressor from *Drosophila*. Expression of this chimeric construct in transgenic plants should displace the native gene product from its target site or titrate interacting proteins and create a mutant phenocopy of loss-of-function alleles. We have shown that this technique can efficiently generate mutant phenocopies for the *STM*, *AP3*, *PI* and *LFY* genes amongst others, and can elucidate the biological function of genes based on gene sequence alone. In addition to being an alternative to conventional methods of mutational analysis, CHRIS technology shows potential to be informative in situations of genetic redundancy and to allow the transfer of the knowledge to other crop species.

7-47 Cell polarity in the Arabidopsis root epidermis requires steady-state sterol cycling

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Plant sterols determine membrane characteristics and serve as precursors for steroid hormones. In contrast to animals, little is known about intracellular sterol trafficking in plants due to the lack of sterol-specific probes. We have visualized intracellular sterol distributions in the *Arabidopsis* root epidermis by employing sterol-specific filipin fluorescence. Sterols mainly localised to the plasma membrane but pharmacological or genetic interference with sterol biosynthesis caused intracellular sterol accumulation. In interphase cells, sterol trafficking strongly depended on the actin cytoskeleton, while microtubules were required for sterol redistribution from the phragmoblast during cytokinesis. Both sterol redistribution from the phragmoblast and sterol trafficking in interphase cells required a brefeldin A (BFA)-sensitive vesicle trafficking pathway. BFA caused co-accumulation of plasma membrane proteins and sterols in endosomes suggesting that their recycling involves a common pathway. Mutations and pharmacological treatments interfering with sterol biosynthesis, plasma membrane localisation, and internalisation caused defects in epidermal polarity. These included aberrations in cell division plane orientation and apical-basal trichoblast polarity. Apparently, sterol cycling comprises a steady-state between sterol biosynthesis, plasma membrane trafficking, and intracellular retrieval and contributes to the execution of cell polarity programmes in the *Arabidopsis* root epidermis.

7-48 Cytogenetic analysis of *hop1, dmc1, mei1* and *dsy1* meiotic mutants of *Arabidopsis thaliana*. A comparative study

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Meiotic analysis was carried out in four meiotic mutants of *Arabidopsis thaliana* using fluorescence in situ hybridization (FISH) techniques at different stages of meiosis.Mutations generated by T-DNA insertional mutagenesis were identified by PCR procedures. The mutants were isolated could be observed that they affected the coding regions of DMC1 and MEI1 genes located on chromosomes 3 and 1, respectively, or the promoter region of HOP1 on the chromosome 1.
Cytogenetic analysis of the first meiotic prophase indicated that the centromeric regions are not located in a specific position of the nucleus in early meiosis. The association of homologous centromeric regions are highly altered in the prophase stages in all meiocytes of *hop1, dmc1* and *dsy1* mutants. However, for *mei1* mutants, a certain cell heterogeneity was found, since cells with and without homologous centromeres associated has been observed. The study of early meiosis stages using telomeric probes has revealed that the telomeric chromosome regions were not persistently associated, and consequently a clustering of telomeres was not clearly detected.
At metaphase I stages it was possible to distinguish all chromosomes of the complement by combining different DNA probes. A great meiotic instability, mainly due to the presence of univalents from diplotene to metaphase I was found in all meiotic mutants analyzed. In *dmc1*, *mei1* and *dsy1* mutants an absence of chromosme associations was found from diplotene to metaphase I, indicating their asynaptic behaviour of these mutants. In addition, *mei1* mutants showed a high degree of chromosome fragmentation. However, *hop1* mutant showed residual meiotic pairing, represented by the presence of rod bivalents at these stages.

7-49 Characterization of the Arabidopsis catalase family

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The Arabidopsis catalase gene family includes three genes (*CAT1, CAT2 and CAT3).* Catalase is a tetramerand catalase activity gels reveal that three isozymes are detectablethroughout the Arabidopsis life cycle. In order to correlate eachisozyme with its respective CAT monomer, we have generated polyclonalantibodies specific for CAT2 and CAT3, and have identified T-DNAknockouts eliminating CAT2 or CAT3 as well as a deletion line lackingboth CAT1 and CAT3 (a gift of Jeff Dangl and J.B. Morel). The mostabundant and highest mobility isozyme corresponds to the CAT2homotetramer, while the CAT3 homotetramer is the lowest mobilityisozyme and is considerably less abundant. We also used ou antibodies for immunolocalization studies, and show that CAT2 isfound in the peroxisomes of all photosynthetic cells, indicating thatCAT2 is the photorespiratory catalase. Nonetheless, the viability ofthe homozygous *cat2*, *cat3* and *cat1cat3* knockoutsdemonstrates that none of the *CAT* genes is essential, at leastunder normal laboratory conditions. We are currently constructing thetriple mutant to test whether any catalase is essential. Finally, wehave characterized the temporal and spatial expression patterns of *CAT2* and *CAT3* with a combination of GUS and GFPfusions, in situ hybridization and northern blots. These results will be discussed in the context of just how much catalase activity, if any, is required for plant viability. This work was supported by grants from the National Science Foundation (IBN 9817603) and the United States Department of Agriculture (NRICGP 9602632).

7-50 A novel alpha-integrin-like protein from *Arabidopsis thaliana*

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In mammalian cells, signaling across a dynamic continuum involving the extracellular matrix, the plasma membrane, and the cytoskeleton involves specific plasma membrane receptors called integrins which recognize a family of extracellular adhesive glycoproteins via the sequence Arg-Gly-Asp (RGD), a motif conserved in each of the adhesive proteins. Integrins are a family of heterodimeric transmembrane proteins formed by the non-covalent association of one polypeptide of fifteen structurally related alpha-subunits with one polypeptide of eight structurally related beta-subunits. The specific recognition system between integrins and the extracellular matrix proteins has been implicated in differentation, proliferation and transmembrane signaling (Gumbiner,1996). Peptides containing the RGD motif (RGD peptides) are competitive inhibitors for the matrix-membrane binding sites at the membrane, and have been used to study the function of these interactions in animal cells.

Evidence for the presence of integrin-like proteins in plants relies on the fact that treatments with RGD peptides influence several plant physiological processes, such as adventitious root formation (Díaz-Sala et al., 2002). In addition, high affinity RGD-binding sites at the *Arabidopis* plasma membrane that link the cell wall have been described (Canut et al., 1998). On the other hand, inmunological data suggests that plants contain proteins related to animal integrins (Laboure et al., 1999), and genes that are predicted to have beta-integrin-like domains have been isolated (Laval et al., 1999, Napgal and Quatrano, 1999).

A novel *Arabidopsis thaliana* gene, called *ALPHA-INTEGRIN-LIKE (AIL*), has been cloned based on homologies to animal alpha-integrins. The cDNA is 2724 bp and has an open reading frame encoding a protein of 907 amino acids. The predicted sequence indicates that it is a transmembrane protein with close-related characteristics to the human alpha8- and alpha9- integrins and to the *C. elegans* vitronectin receptor. Among other features, it contains the EF-hands and the glycosilation sites typical from alpha-integrins. RT-PCR studies show that *AIL* expression is developmentally regulated.

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7-51 *RHD6* is a positive regulator of root hair cell development

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Root epidermal cell fate in *Arabidopsis* is under the control of a transcription factor cascade involving *GL2, TTG, WER and CPC*. The default state of an epidermal cell is to produce a root hair. Epidermal pattern is achieved by the spatial expression of *GL2* in non hair cells where it negatively regulates root hair production. This spatial expression of *GL2* is controlled by the upstream activity of *TTG, WER and CPC*.
The mutation *rhd6-2* was isolated from an enhancer-trap mutagenesis. Plants homozygous for the mutation had roots with a reduced number of hairs. This implies the *RHD6* gene is required for normal root hair development. This study examines the effect of the mutation, the expression pattern of the gene using the GUS marker gene and the genetic interaction of the gene with other genes.
In *rhd6-2*, cells specified as hair cells produced 0-3 short hairs with regular mis-localisation of the initiation site. *RHD6* is expressed only in cells in the root hair position. Double mutant analysis revealed that *TTG, WER* and *CPC* act upstream of *RHD6*. Analysis of GUS expression indicated that *TTG* and *WER* negatively regulate *RHD6* in non hair cells and *CPC* activity is required for *RHD6* expression in hair cells. Results imply *RHD6* has a role early as a positive regulator of hair cell fate and also has a role later during hair initiation and growth.

7-52 Functional characterization of Arabidopsis S-like RNases

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Extracellular and vacuolar ribonucleases (secretory RNases) have been well-studied at the enzymatic and structural levels. However, little is known regarding their biological functions. One family of secretory RNases, the RNase T2 family, is particularly widespread. Expression patterns and cellular localization suggest that these enzymes could be involved in nutrient recycling. A defined biological role has only been described for the S-RNases, a plant subfamily involved in the cytotoxic process during self-incompatibility. S-like RNases, another plant subfamily, are found in self-compatible and -incompatible plant species.
To understand the biological roles of the T2 family, we constructed an insertional mutant of RNY1, the only member of this family in yeast. RNY1 encodes an extracellular RNase whose expression is regulated by stress conditions, including heat shock and osmotic stress. Inactivation of Rny1 leads to the formation of large cells that are osmo- and temperature-sensitive and flocculate in culture. These phenotypes can be complemented by both structurally related and unrelated secetory RNases. Complementation is dependent on RNase activity. Our studies in yeast are complemented by those of RNS1 from *Arabidopsis thaliana*. This extracellular enzyme is induced by Pi-starvation, indicating a role in Pi remobilization. However, RNS1 is also induced by wounding, both locally and in distal, undamaged leaves, suggesting additional functions for RNS1, possibly involving defense responses. Arabidopsis mutants that lack RNS1 activity show an increase in growth that is particularly evident in roots. This phenotype and the regulation patterns of RNS1 suggest that T2 RNases in yeast and plants may have similar functions.

7-53 Organisation and assembly of the secondary cell wall cellulose synthase complex

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Significant advances have recently been made in the cloning of genes involved in cellulose synthesis. Despite the identification of these genes, the structure of the cellulose synthesising rosettes has not been elucidated. Using Arabidopsis mutants deficient in secondary cell wall cellulose synthesis, a number of *irx* (*irregular xylem*) complementation groups have been identified (Turner and Somerville 1997). *IRX3* and *IRX1* have been cloned and were found to encode distinct members of the CesA gene family predicted to encode cellulose synthase catalytic subunits (Taylor *et al* 1999, 2000). A further novel complementation group, *irx5*, has been identified and the corresponding gene cloned. *IRX5* encodes another member of the CesA gene family which is essential for secondary cell wall cellulose synthesis. IRX1, IRX3 and IRX5 have been shown to interact as part of the same complex. In addition, in the absence of IRX5, IRX1 and IRX3 no longer associate. This suggests an essential role for all three CesA proteins in the assembly of the secondary cell wall cellulose synthesising complex. The organisation and stoichiometry of the subunits within the complex is currently being studied by native electrophoresis and purification of the complex using a novel hexa-His tagged IRX3 line. These approaches will also allow us to identify other proteins associated with the rosettes involved in the synthesis of cellulose.

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7-54 Dynamic interaction between chloroplasts and actin filaments depend upon the participation of an actin-like protein

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Due to their non-motile nature, plants have evolutionarily adapted to changes in the environment by modifying their growth and metabolism. Despite the non-motility of plant cells, the organelles within them move intra-cellularly in response to environmental and developmental stimuli. One example of this intra-cellular relocation is the repositioning of chloroplasts in response to light stimuli. Previous reports have indicated that this repositioning may be an actin based process. However, a direct interaction has not previously been demonstrated. We have been able to reconstitute chloroplast-actin interaction in vitro. Isolated chloroplasts are capable of nucleating F-actin. Our results also indicate that there is an actin-related protein associated to isolated cloroplasts that may play a role in this interaction.

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7-55 *HYP6* and *HYP7*: Two Arabidopsis genes required for normal cell expansion

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In order to advance our understanding on plant cell expansion, we have conducted a screen to look for Arabidopsis mutants with reduced hypocotyl elongation and identified two new mutants, hypocotyl 6 (hyp6) *and hypocotyl 7 (hyp7*). In the subsequent morphological characterisation, we found that these mutants are defective in cell expansion in many other tissues, including trichomes, root hairs and various parts of mature plants. This suggests that HYP6 and HYP7 are essential for both diffuse and tip growth of cells. We found that the microtubule arrays in both mutants are normal. Fourier transform infrared (FTIR) microspectroscopy indicated that the cell walls of the mutants have normal levels of cellulose but contain more pectic galacturonate than wild-type plants. Sugar and methylation analyses and immuno-localisation with antibodies that recognise cell wall epitopes also showed changes in matrix polysaccharide composition. Despite having normal cellulose content, the alignment of microfibrils in the mutants is disrupted from the wild-type transverse array. Despite the hypocotyl being thinner than wild-type, the tensile strength of this organ is normal but the tensile modulus is much reduced. We conclude that normal cellulose alignment is not necessary for organ strength but this and other cell wall changes affect the elasticity of the organ. These two mutants provide new tools with which to dissect the key processes of cellular and plant growth.

7-56 Integration of cell cycle control and differentiation: Function of the Retinoblastoma-related protein RBR in *Arabidopsis thaliana*

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The Retinoblastoma (Rb) protein is critical in the regulation of cell cycle progression, especially at the G1/S transition. The activity of Rb in mammals is dependent on its phosphorylation state. When hypo-phosphorylated, it binds to the E2F family of transcription factors and blocks transcription of S phase-specific genes. Following phosphorylation of Rb by cyclin D-CDK complexes, E2F is released and activates transcription for progression into S phase. In addition, Rb interacts with developmental transcription factors and chromatin-modifying complexes, suggesting a role for the protein in development.

Retinoblastoma-related proteins (RBR) have been isolated from different plants. In Arabidopsis, RBR is encoded by a single gene. The structure of the protein is well conserved between mammals and plants. But it is still unknown to what extent plant RBR proteins are functionally similar to their mammalian counterparts. To dissect the function of Arabidopsis RBR1, we are employing ectopic expression and RNA interference strategies using the CaMV 35S promoter and cell/tissue specific promoters. *RBR1-RNAi* gene constructs expressed under the control of the *WUS*, *STM or CLV1* promoters produce significantly different phenotypes, suggesting that RBR1 has distinct functions in specific domains of the meristem. We have also screened several T-DNA collections for insertions in *AtRBR1*. Three lines have been identified with insertions in the promoter region, the 5'UTR and near the 3' end of the coding region, but it appears to be difficult to obtain insertions in other regions of the large (> 6 Kb) gene.

7-57 Redox-mediated induction of poising systems in *Arabidopsis thaliana*

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There is increasing evidence that the redox pressure in the photosynthetic electron transport chains of chloroplasts controls the expression of several nuclear-encoded genes, but it is still not clear which genes are regulated by this way. For the identification of possible candidates, the electron pressure in chloroplasts of low-light acclimated A. thaliana plants was altered by combinations of (i) increasing the light intensity, (ii) lowering the temperature (12°C) and (iii) limiting the supply of photosynthetic acceptors (O2 and CO2) by diluting the surrounding air with N2. Rapid changes in both, protein and mRNA amount of several enzyme systems involved in maintaining the redox state of the cell occurred within several hours. The amount of NADP-malate dehydrogenase, the rate-limiting enzyme of the malate valve, started to increase after 4-6 hours. Under strong acceptor limitation, its amount increased 10-fold within 4 days. The mRNA levels of most enzymes involved in glutathione synthesis increased with the same time course. In parallel, the contents of free and protein-bound glutathione in the leaves increased. After 4 days, the amount of protein-bound glutathione was doubled. The amount of free glutathione increased 4-fold, while the redox state of the glutathione pool remained unchanged. Interestingly, the expression of other antioxidative enzymes such as catalase or ascorbate peroxidases, and of several Calvin-cycle enzymes remained constant under most conditions. The results indicate that NADP-malate dehydrogenase and the enzymes of glutathione metabolism are good candidates for targets of redox-regulated gene expression.

7-58 Arabidopsis mutants altered in alpha-fucosidase and alpha-xylosidase able to act on xyloglucan side-chains

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The cellulose-xyloglucan complex is the load-bearing network in the type I cell walls, as the one from *Arabidopsis thaliana* are. Xyloglucans (XGs) interact with cellulose microfibrils via hydrogen bonds and thus play an important role in cell wall expansion during plant development. The core XGs have a backbone of beta(1,4)glucan chain branched in a regular pattern with alpha-xylosyl and alpha-fucosyl-beta-galactosyl-alpha-xylosyl residues. In addition to its structural role, xyloglucan may also act as a source of signalling molecules, oligosaccharides derived from XG. The structure of the side-chains of XG has been related with the ability of XG to bind to cellulose microfibrils. Moreover, in the case of XG-oligos, it has been also related with the biological activity. Thus, the apoplastic enzymes that modify the side chains of XG and/or of XG-oligos are good candidates to play a regulatory role in the dynamics of XG-cellulose network. We have already identified in *Arabidopsis* two genes *AtFXG1* and *AtXYL1* coding for alpha-fucosidase (Sampedro et al., 2001) and alpha-xylosidase (Torre et al., 2002), respectively. With this in mind we look for mutants altered in both mentioned activities. Screening of *Arabidopsis* mutant collections produced two mutants, *Atfxg1* and *Atxyl1*, with an insertion within the coding region of the *AtFXG1* and *AtXYL1*, respectively. The mutants lack alpha-fucosidase and alpha-xylosidase activities, respectively. Detailed characterization of both mutants in currently under way and it will be presented.

Sampedro et al. 2001. Plant Physiol. 126: 910-920
Torre et al. 2002. Plant Physiol. 128 : 247-255

7-59 Fast and continuous elongation control in Arabidopsis roots: Cell elongation is controlled by multiple factors

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The fast regulation of elongation rate by ethylene greatly relies on the extent of cell elongation in the elongation zone proper. The positive or negative regulation of cell length with 2-aminoethoxyvinylglycine (AVG), an ethylene synthesis inhibitor, and 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene, is associated with transverse or longitudinal oriented microtubules, respectively. As soon as ethylene stops cell elongation, cortical microtubules change their orientation from transverse to longitudinal within 1 hour. Experiments combining the effects of ACC, microtubule drugs (oryzalin and taxol) and osmotic stress indicate that reorientation of microtubules is not causal to but concomittant with the inhibition of elongation. The same can be said for actin filaments (F-actin), the other cytoskeletal component, essential for cell elongation. It is thus not the orientation of cellulose microfibrils that is the responsive factor in fast control of cell elongation and wall extension. AVG or ACC also do not change the profile of high xyloglucan endotransglucosylase/hydrolase (XTH) action along the elongation zone; they only slightly change its peak intensity. However the cellulose synthesis inhibitor, 2,6-dichlorobenzonitrile (DCB) rapidly affects cell elongation with a response time similar to that of the ethylene effect. Also it was demonstrated that XTH action related to elongation is intimately linked to the presence of nascent cellulose fibrils. Together, these results suggest that the fast control of wall extension implies complex changes in wall´s polymer networks.

7-60 Arabidopsis Transportin1 is the nuclear import receptor for the circadian clock-regulated RNA-binding protein AtGRP7

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We characterized a novel nuclear import pathway in *Arabidopsis thaliana*. Transportin 1 (AtTRN1) is the nuclear import receptor for heterogeneous nuclear ribonucleoproteins (hnRNPs) of plants. AtTRN1 also interacts with hnRNP A1 from humans and with Nab2p from yeast. Like all members of the Importin beta family of transport receptors, it interacts with the regulatory GTPase Ran. AtTRN1 conferred nuclear import of fluorescently labeled BSA-M9 peptide conjugates in permeabilized HeLa cells, functionally replacing human Transportin 1. We identified three plant proteins that interact with Arabidopsis Transportin 1 and that contain M9-like domains: a novel Arabidopsis hnRNP that shows high similarity to human hnRNP A1 and two small RNA-binding proteins from Arabidopsis, AtGRP7 and AtGRP8. Nuclear import activity of the M9-like domains in these plant proteins was demonstrated by a nuclear export competition assay. Fluorescently labeled AtGRP7 was specifically imported into the nuclei of permeabilized HeLa cells by Arabidopsis and by human Transportin 1. Since AtGRP7 has been characterized as a component of a slave oscillator to the circadian clock in Arabidopsis the Transportin-mediated nuclear import of AtGRP7 may be a component of this oscillatory mechanism.

7-61 Characterization of Arabidopsis H^+-pump ATPase (*AHA*) mutants

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Most nutrient and metabolite transport systems in plant cells utilize the energy of the electrochemical gradient of protons across the plasma membrane. Plasma membrane proton–transporting ATPases generate this proton gradient at the expense of ATP hydrolysis. The *Arabidopsis* H+ pump ATPases (AHAs) family includes twelve genes, suggesting that some AHAs may have redundant activities, and/or some AHAs are specialized for specific physiological functions. Previous studies have indicated that *AHA* and *AHA2* are expressed in roots, and *AHA3* is expressed in the phloem and reproductive organs. We are using a reverse-genetics approach to elucidate the function of AHAs in Arabidopsis. Preliminary analysis of plants homozygous for T-DNA insertions in *AHA2* showed that *aha2* mutant seedlings have growth deficiencies when grown in the absence of sucrose or low pH. While individual *aha1* and *aha2* mutants have noticeable phenotypes but complete their growth cycle, genetic analysis show that double homozygote mutants are not viable. Thus, the combination of null alleles of *AHA1* and *AHA2* is lethal. Similarly, exhaustive screens for plants with insertions in *AHA3* have identified multiple alleles of *aha3*, but no homozygous *aha3* mutants, indicating that functional AHA3 is required for development. The lethal effect of mutations in AHA genes indicate that AHAs play an essential role in plant growth and development. A further characterization of the effects of mutations in AHAs and the specific cause of lethality caused by the mutations is in progress.

7-62 RNA interference of reproductive actin expression in Arabidopsis

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Actin is a major cytoskeletal component of the cell. Within plant cells, actins play highly influential roles in cell polarity, root and pollen tube tip growth, cellular scaffolding, organellar positioning and movement, and cell division. Five reproductive and three vegetative actin genes compose the eight differentially expressed members of the *Arabidopsis* actin family. The reproductive actins are predominantly expressed in pollen, pollen tubes, ovules, and developing embryos in contrast to the vegetative actins present in all tissues except pollen sacks and ovules. Mutants were isolated and created to gain understanding of the significance of multiple, differentially expressed actins present throughout the reproductive tissues. ACT11 was the first reproductive actin targeted for mutagenesis, because of its unique pattern of expression in both pollen and ovules, and in early embryonic and seedling development. Interference RNA was targeted to the 3' UTR of *ACT11* to distinguish it from other members of the actin gene family. Initial characterization of these ACT11 RNAi-suppressed mutants revealed a delay in germination, smaller young seedlings, and reduced hypocotyl length in etiolated seedlings. Putative phenotypes include reduced embryogenesis and shriveled carpal walls. Western analysis also demonstrated reduced actin protein expression in reproductive tissues. Immunofluorescence of the microfilaments, 2D gel analysis, and characterization of two *ACT11* T-DNA insertions from a T-DNA collection will be critical in deciphering the significance of the tissue-specific expression of diverse actin isovariants.

7-63 New tonoplast-associated proteins are likely involved in membrane fusion in Arabidopsis

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The biogenesis of plant vacuoles is well understood at the morphological level, but the molecular machinery that is involved in vacuole formation is still unknown. Recently we have identified an embryo-lethal mutation in Arabidopsis, *vacuoleless1 (vcl1)*. The VCL1 protein shares significant homology with Vps16p from yeast, which is part of the C-VPS complex that also includes Vps11p, Vps18p, Vps33p, Vps39p, and Vps41p. In yeast, this complex is required for homotypic fusion of vacuoles and heterotypic fusion of vesicles at the tonoplast, and contributes to the specificity of these processes. The components of the C-VPS complex are conserved in plants, fungi, and animals. In Arabidopsis, a single gene homologue for each component has been identified. In this work, we report that VCL1, AtVPS33, and AtVPS11 are peripherally associated with membranes and have similar protein expression patterns in plant tissues. We also provide evidence that VCL1 interacts directly with AtVPS33. Based on immunoelectron microscopy, both AtVPS33 and AtVPS11 colocalize to the tonoplast, supporting the role of the AtC-VPS complex in regulating membrane fusion.

7-64 Isolation of an Arabidopsis T-DNA mutant for the chloroplast protein import receptor AtToc33

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A great number of plastid proteins are nuclear encoded, are synthesized in the cytosol as precursor with a N-terminal extension (transitpeptide) and have to be imported into the organelle posttranslationally. Two protein complexes located in the plastidic envelope membranes are responsible for this transport process. One of the first steps of this transport is the binding of the precursor protein with its transitpeptide to one or several receptor proteins, that are exposed on the cytosolic surface of the plastids. Toc34, a GTP binding component of the import complex in the outer chloroplast envelope, is one of these receptors and was shown to directly interact with precursor proteins. To gain more insight into the in vivo function of this receptor a reverse genetic approach was taken. A collection of about 32000 Arabidopsis T-DNA lines was sucessfully screened for insertions in the atToc33 gene, one of the two Arabidopsis Toc34 homologues. The T-DNA insertion in the isolated mutant is located in the promotor region of the atToc33 gene. The atToc33 mutant shows a pale yellowish phenotype, that has also been observed after antisense repression of atToc33, and could be complemented upon transformation with the wildtype cDNA. Further molecular and physiological characterisation of the mutant will be presented.

7-65 Functional analysis of AtVTI1, a family of SNARE proteins in Arabidopsis

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Proteins destined for the vacuolar compartment contain targeting signals in their primary sequences. Proteins that carry N-terminal pro-peptide signals (NTPPs) are sorted by NTPP receptors at the trans-Golgi network (TGN) and packaged into clathrin-coated transport vesicles. The vesicles are transported to the pre-vacuolar compartment (PVC), which subsequently fuses with the vacuole. Correct targeting of NTPP vesicles to the PVC most likely involves SNARE proteins.
In order to understand the vacuolar trafficking step between the TGN and PVC, we have isolated two related SNARE genes from Arabidopsis: *AtVTI11* and *AtVTI12*. AtVTI11 and AtVTI12 share a high degree of sequence similarity (65% identical at the amino acid level). However, they complement different yeast *vti1* mutants and in Arabidopsis, are located on different organelles in the endomembrane system. AtVTI11 is localized on the domain of the TGN and forms SNARE complexes with SYP2 and SYP5. These data suggest that AtVTI11 is a SNARE involved in directing NTPP cargo-containing vesicles towards the PVC. AtVTI12 is localized to a different domain of the TGN, where it forms a SNARE complex with SYP4 and SYP6.
We are examining two mutant lines that lack AtVTI11 and AtVTI12. The gravitropic response mutant *zig*-1 has been shown to be defective in *AtVTI11*. We have identified a T-DNA insertion in *AtVTI12*. In the *Atvti12* mutant, co-immunoprecipitation experiments have shown that AtVTI11 forms a SNARE complex with SYP4 and SYP6, and therefore serves as functional substitute for the VTI12 protein. We will also present data from the double mutant cross of *Atvti12* and *zig-1*.

7-66 Oryzalin hypersensitive mutants of Arabidopsis

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It has long been known that there is a connection between the cell wall and the microtubule cytoskeleton. Cellulose is deposited perpendicular to the direction of cellular expansion, directionally reinforcing the cell wall and directing turgor driven expansion. Disorganization of cortical microtubules with microtubule destabilizing drugs causes disorganized cellulose deposition and isometric cellular swelling. Cortical microtubules are thought to guide cellulose deposition because there is a correlation between the orientation of cortical microtubules and cellulose microfibrils. In order to find the components regulating cortical microtubule dynamics and cellulose deposition, I have isolated Arabidopsis mutants that are hypersensitive to the microtubule-destabilizing drug oryzalin. From a large-scale screen, over 130 mutants have been recovered and are being characterized. Several mutant lines have defects suggestive of abnormal cytoskeletal organization, such as root skewing and cold sensitivity. Direct examination of the microtubule cytoskeleton is underway.

7-67 A gene family in *Arabidopsis thaliana* involved in magnesium transport

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Three genes from a single gene family in *Arabidopsis thaliana* have been shown to complement magnesium (Mg) transport across biological membranes in exogenous mutant systems (Schock et al., 2000 [*Saccharomyces cerevisiae*], Li et al., 2001 [*S. cerevisiae, Salmonella typhimurium*]). Seven additional members of the family were identified using the public *Arabidopsis* genomic sequence database. The genes are expressed in a range of tissues in *Arabidopsis* (Li et al., 2001). Full-length cDNA copies of the genes have been cloned and sequenced and a phylogeny constructed from the predicted protein sequence (Li et al., 2001). All of the family members have been over-expressed in two yeast strains using the yeast expression vector pFL61 (Minet et al., 1992). The first strain (CM52) is nominally wild type, while the second (CM66) is derived from CM52 having had the two Mg uptake genes (*ALR1* and *ALR2*) deleted. The level of complementation (restoration of growth on media containing normal levels of Mg) in CM66 by each of the genes has been characterised and will be reported. To further understand the phenotypes of the CM66 yeast over-expressing the family we are currently trying to localise the proteins within the yeast cell. To date we have fused GFP to the C-terminus of eight members of the family. Initial microscopy of the yeast expressing the fusion proteins shows a range of GFP intensities and localisations. We are currently testing the fusion constructs ability to complement the Mg uptake deficiency.

7-68 Functional diversity of Arabidopsis homologues of the Rab1/Ypt1 GTPase subclass (AtRabD)

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Rab GTPases are small key regulators of intracellular membrane trafficking.The Arabidopsis Rab gene family is relatively large. The 57 members are assigned to just 8 subclasses on the basis of sequence comparisons. In contrast to other eukaryotes, each subclass contains multiple isoforms, of which the level of functional redundancy is unclear. The aim of this work is to investigate whether two isoforms of the Arabidopsis RabD subclass (homologous to Rab1 /Ypt1), AtRabD1 and AtRabD2a, perform distinct cellular functions. We have usedtransient expression in tobacco leaf epidermis to show that dominant negative forms of AtRabD1 and AtRabD2a cause inhibition of the transport of a secreted form of Green Fluorescent Protein (secGFP), and a quantifiable accumulation of GFP within the endoplasmic reticulum (ER). This suggests that both members of the AtRabD subclass may be involved in regulation of transport between the ER and the Golgi apparatus. The inhibition of secGFP transport caused by expression of the dominant negative form of the Rab may be rescued by coexpression of the wild type form of the same Rab. Reciprocal rescue tests show that AtRabD1 and AtRabD2a are unable to functionally substitute for each other in this assay, suggesting that the two RabD proteins may act on vesicle traffic via distinct biochemical pathways.

7-69 Expression analysis of the 19 Arabidopsis *SKP1-like* genes

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The yeast Skp1p is a component of the SCF complex, an E3 enzyme involved in the specific protein degradation pathway *via* ubiquitination. *SKP1-like* genes have been found in a variety of species including yeast, human, *C. elegans* and *A. thaliana*. The Arabidopsis genome contains 19 *SKP1-like* genes called *ASK* (for Arabidopsis SKP1-like) among which *ASK1* has been well characterized. The *ask1-1* mutant phenotype implies that *ASK1* is active only in sporophytic tissues. Recently, we identified a new member of the *SKP1*-like gene family in rapeseed. This gene, called *BnSKP1g1*, is expressed in both male and female gametophytes, unlike *ASK1*. As nucleotide and protein sequence analysis did not allow the identification of an *A. thaliana* ortholog of *BnSKP1g1*, we compared the expression patterns of *ASK* promoters-*GUS* fusions with the one of *BnSKP1g1*. Four *ASK* promoters are found to trigger no *GUS* expression either in inflorecences nor in seedlings. The *ASK1* expression profil observed in consistent with previous findings. Surprisingly, we found that 9 *ASK* promoter-*GUS* fusions show expression in the male gametophyte (after meiosis), among which two are expressed in both male and female gametophytes. The corresponding *ASKs* may thus be the potential orthologs of *BnSKP1g1*. Moreover, some *ASK* promoters localise specific expression as for instance in the connective of anther or the embryo.

7-70 GTPases in the outer chloroplast envelope and their role in protein import

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The import of nucleus-encoded proteins into chloroplasts is facilitated by multimeric translocation complexes in the outer (Toc complex) and inner (Tic complex) envelope membranes. In pea, preprotein recognition is controlled by two homologous GTPases, Toc34 and Toc159, which are activated by dephosphorylation and GTP binding. In *Arabidopsis* there are two *Toc34* genes, *Toc33* and *Toc34*. *Toc33* is expressed at 5-fold higher levels than *Toc34* and is the direct orthologue of pea *Toc34*. The *Arabidopsis Toc33* knockout mutant, *ppi1*, has a pale phenotype and aberrant chloroplasts with reduced protein import capabilities. Chloroplast proteome analysis of *ppi1* revealed many changes, with a number of proteins either deficient (for example: Rubisco SSU and OE33) or enriched in *ppi1* compared to wild type. We have been investigating the import defect of *ppi1* in detail using a range of different precursors. Import rates of photosynthetic proteins preSSU and preOE33 were strongly reduced in *ppi1*, whereas the import of ribosomal protein preL11 was not affected, suggesting that Toc33 is involved in the import of only a subset of precursors. The analysis of additional preproteins in further import experiments is being used to confirm this apparent precursor protein recognition specificity. Additionally, *Arabidopsis* knockout mutants lacking the Toc159 homologues (Toc132, Toc120 and Toc89) have been generated and are currently being characterized.

7-71 Variations in sorting pathways for peroxins and other peroxisomal membrane proteins

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Peroxin genes (*PEX*) code for a set of proteins that participate inperoxisomal biogenesis. Fifteen Arabidopsis orthologs of 23 yeast*PEX* genes have been identified. Most (11) are peroxisomal membrane proteins (PMPs), and functions are known for only four of them. Also, approximately 25 non-peroxin PMPs have been identified. All peroxins and non-peroxin PMPs are synthesized on cytosolic polysomes; whether they sort directly to peroxisomes from the cytosol, or indirectly via ER or vesicles is a main focus of our research.
Data were obtained from cell fractionation, or immunofluorescence microscopy, of suspension-cultured Arabidopsis and/or BY-2 cells. Overexpressed CvPex5p and AtPex7p were exclusively cytosolic, where they function as matrix protein receptors, possibly in the extended shuttle pathway. The AtPex16p ortholog was interpreted in a genetic study of Arabidopsis plants to function in protein and oil body biogenesis. In contrast, we found that AtPex16p sorted directly to peroxisomes. Pex10p exists in yeast and mammalian peroxisomal membranes, whereas the AtPex10p ortholog is not in plant peroxisomes, but is in rough ER and smooth vesicles where it may function as an early peroxin. Peroxisomal ascorbate peroxidase (APX) sorted indirectly to peroxisomes via a peroxisomal ER subdomain. AtPex10p and APX were in ER of non-transformed cells, thus results are not artifacts of overexpression. Peroxisomal membrane MDAR (54, not 32 kD) also seems to sort through ER. In summary, PMPs sort to peroxisomes via varied pathways; the means of selective sorting is not yet elucidated.

7-72 Genetic clues to unravel the cpSRP pathway for the targeting of LHC proteins

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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 a-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 athway. 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.

8-01 Genetic approaches confirm an important role for *CBFs* in cold acclimation and reveal a complex network for regulating *CBF* expression

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Many plants increase in freezing tolerance upon exposure to low nonfreezing temperatures, a process known as cold acclimation. Genetic analysis indicate that the ability to cold acclimate is a quantitative genetic trait. Consistent with its multigenic character, the process of cold acclimation involves numerous biochemical and physiological changes, most of them regulated at the gene expression level. Studies on *Arabidopsis* cold-regulated gene expression allowed to identify a family of transcriptional activators (CBF1-3) that bind to the DRE/CRT regulatory element (CCGAC) present in the promoters of some cold- and drought-inducible genes. Overexpression of the *CBF* genes in *Arabidopsis* induces the expression of several cold-inducible genes and increases freezing tolerance, which suggests that they play an important role in cold acclimation response. Analysis of natural variation for freezing tolerance in the *Arabidopsis* ecotypes Ler and Cvi seems to confirm this role. Importantly, the *CBF* genes themselves are also induced by low temperature, but they do not appear to be autoregulated. Results obtained from a screening for mutants affected in *CBF2* expression suggest the existence of a complex network of pathways involved in *CBF* regulation.

8-02 Identification of genes controlling osmotic tolerance by large scale screening of Arabidopsis T-DNA insertion mutants

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To identify the genetic loci controlling salt tolerance in higher plants, large scale screens were conducted with a bialaphos marker based T-DNA insertional collection of *Arabidopsis thaliana* mutants. One particular line *osm1* (for osmotic sensitive mutant) exhibited increased sensitivity to both ionic (NaCl) and non-ionic (mannitol) osmotic stress in a root-bending assay. The *osm1* mutant displayed a more branched root pattern with or without stress and was hypersensitive to inhibition by Na+, K+, Li+, but not Cs+. Plants of the *osm1* mutant also were more prone to wilting when grown with limited soil moisture compared to wild type plants. The stomata of *osm1* plants were insensitive to both ABA-induced closing and inhibition of opening compared to wild type plants. The T-DNA insertion appeared in the promoter region of an open reading frame (ORF) on chromosome 1 (*F3M18.7*, same as *AtSYP61*). This insertion mutation co-segregated closely with the osm1 phenotype and was the only functional T-DNA in the mutant genome. Expression of the *OSM1* gene was disrupted in mutant plants and abnormal transcripts accumulated. Gene complementation with the native gene from the wild type genome completely restored the mutant phenotype to wild type. Analysis of the deduced amino acid sequence of the affected gene revealed that OSM1 is most closely related to mammalian syntaxins 6 and 10, which are members of the SNARE superfamily of proteins required for vesicular/target membrane fusions. Expression of the *OSM1* promoter::*GUS* gene in transformants indicated that *OSM1* is expressed in all tissues except hypocotyls and young leaves and is hyperexpressed in epidermal guard cells.

8-03 Arabidopsis mutation *dry1* identifies a gene essential for drought tolerance

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Osmotic stress caused by drought is one of the most detrimental environmental stress condition limiting plant productivity. One strategy to identify genes essential for osmotic tolerance is to identify mutant plants hypersensitive to osmotic stress. Because in the search for NaCl hypersensitive mutants, only ionic mutants have been identified, we employed mannitol in our screening in order to identify osmotic hypersensitive mutants. Screening of 45.000 EMS-mutagenized seeds from Arabidopsis thaliana genotype Landsberg erecta resulted in the isolation of a recessive mutant denominated *dry1* (for drought hypersensitive). Root growth of *dry1* is specifically hypersensitive to osmotic stress caused by mannitol, sorbitol, choline chloride and proline but is not hypersensitive to NaCl. Analysis of the proline content indicated that *dry1* is not defective in proline accumulation upon osmotic stress. Similarly, root growth of *dry1* at various abscisic acid (ABA) concentrations and the endogenous ABA content were not affected in the mutant. Adult *dry1* plants were hypersensitive when drought was applied slowly. However no differences in hypersensitivity between WT and *dry1* were found when drought was applied drastically. These data suggest that long-term adaptation to osmotic stress is affected in *dry1*. Northern blot analysis of several osmotic responsive genes showed that *PR5* and *P5CS* were up-regulated in *dry1* respect to wild type after osmotic stress. In contrast *DREB2A* and *RD29A* were down-regulated. We are currently map-based-cloning *Dry1* in order to gain further insight into the role of this gene in osmotic tolerance.

8-04 Isolation of *Arabidopsis* mutants altered in ABA-induction of *rd29B* gene expression

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To dissect the transcriptional regulation and signal transduction of ABA, we isolated *Arabidopsis* mutants altered in their responses to ABA. We introduced a chimeric gene construct consisting of the luciferase (*LUC*) under the control of the ABA-responsive *rd29B* promoter (*rd29B-LUC*) into *Arabidopsis* plants. Activation T-DNA tagged lines of *Arabidospis* containing *rd29B-LUC* were obtained by transformation; mediated by *Agrobacterium* carrying the activation tagging vectors pPCVICEn4HPT or pSKI015. We screened mutants altered in the ABA-response of the *rd29B-LUC* gene during germination with ABA or under the stress conditions in mature plants. Some mutants altered in the regulation of the *rd29B-LUC* gene were identified. Three mutants showed higher expression of *rd29B-LUC*. Physiological, molecular, and genetic analysis of the mutants is in progress.

8-05 Plant salt stress tolerance determinants identified by insertional T-DNA tagging

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Plant salt tolerance and yield stability requires numerous adaptive processes that must be coordinated in function within and between cells and tissues. We have begun an initiative to identify salt tolerance determinants by screening for mutations that alter NaCl sensitivity of plants in T-DNA tagged *Arabidopsis thaliana* populations. Namely, mutations have been identified that alter the salt stress sensitivity of C24 or of Col-0 *gl1 sos3-1* plants. *sos3-1* disturbs ion homeostasis that results in Na+ hypersensitivity. To date, more than 85 mutations in C24 were identified that inhibit seedling growth on medium supplemented with 140 to 180 mM NaCl. The mutations differentially affect responsiveness of plants to Na+/Li+, K+, Cl- or mannitol stress. *stt3-1* mutation in the gene encoding an oligosaccharyl transferase causes Na+, K+ and mannitol but not Li+ sensitivities. A mutation (*npct1*) in a gene encoding a Na+-Pi transporter-like protein effects Cl- sensitivity. Mutations that suppress or increase the NaCl hypersensitivity of *sos3-1* have been identified. AtHKT1 disruption suppresses salt sensitivity of *sos3-1* providing evidence that it is involved in the control of Na+ entry into plant roots. NaCl hypersensitive mutations (cause greater salt hypersensitivity than *sos3-1*) are being characterized.

8-06 Combinatorial interaction of cis elements direct the expression of the Arabidopsis *AtHsp90-1* gene

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The Arabidopsis *hsp90* gene family consists of seven members. In order to determine how the expression of this bona fide AtHsp90-1 cytosolic member is regulated, a comprehensive quantitative and qualitative promoter deletion analysis was conducted. The promoter regions were fused to GUS, the constructs were incorporated into Arabidopsis genome and the expression of GUS was determined under various environmental conditions, and during development. The promoter region of the *Arabidopsis thaliana AtHsp90-1* gene is congested with heat shock elements (HSEs) and stress response elements (STREs), CCAAT/enhancer-binding protein element (C/EBP), metal regulatory element (MRE) and the animal proto-oncogene activating protein 1 element (AP-1). The full-length promoter induces gene expression at high levels both after heat-shock and arsenite treatment, and GUS activity was prominent in all tissues. Nevertheless, progressive deletion of the promoter decreases the level of expression under both treatments and restricts it predominantly in the two meristems of the plant. Nevertheless, under arsenite induction proximal promoter sequences induce AtHsp90-1 gene expression only in the shoot meristem. Under unstressed conditions, distally located elements negatively regulate AtHsp90-1 gene expression. Whereas flower-specific regulated expression in mature pollen grains suggests prominent role of the AtHsp90-1 in pollen development. Our results show that the two stress responses may involve common but not necessarily the same regulatory elements. While for heat induction most elements present in the promoter region facilitate cooperatively in order to promote high levels of gene expression, arsenite induction seems not to be transduced via the STRE and C/EBP elements. The results show that the stress induction or suppression and the regulation of expression during development is mainly due to combinatorial contribution of the cis elements located in the promoter region of the *AtHsp90-1* gene.

8-07 The short-chain alcohol dehydrogenase ABA2 catalyzes the conversion of xanthoxin into abscisic aldehyde

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Mutants able to germinate and carry out early growth in medium containing a high NaCl concentration were identified during the course of two independent screenings and named either *salt resistant* (*sre*) or *salobreño* (*sañ*). The *sre* and *sañ* mutants were also able to germinate in high osmoticum media, indicating they are osmotolerant in a germination assay. Complementation analyses revealed that *sre1-1*, *sre1-2*, *sañ3-1*, and *sañ3-2* were alleles of the abscisic acid (ABA) biosynthesis *ABA2* gene. A map based cloning strategy allowed the identification of the *ABA2* gene and molecular characterization of four new *aba2* alleles. The *ABA2* gene product belongs to the family of short-chain alcohol dehydrogenases, which are known to be NAD- or NADP-dependent oxidoreductases. Recombinant ABA2 protein produced in *E. coli* exhibits a Km value for xanthoxin of 19 μM and catalyzes, in a NAD-dependent manner, the conversion of xanthoxin into abscisic aldehyde, as determined by high performance liquid chromatography (HPLC)-mass spectrometry (MS). The isolation of ABA-deficient mutants by virtue of their capability to bypass inhibition of seed germination and early seedling growth under low water potential conditions highlights an important role of ABA in seed development, i.e. delaying germination and preventing seedling establishment under unfavorable water conditions.

8-08 *FRD3* controls 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 and overaccumulates iron, manganese, and zinc. The *FRD3* gene is expressed in roots and is predicted to encode a membrane protein belonging to the multidrug and toxin efflux (MATE) family. There are two models that most easily explain the phenotype of *frd3* mutants. First, *frd3* mutant plants could have an iron signaling defect. Specifically, *frd3* plants might be unable to sense iron levels, communicate information about iron status between various parts of the plant, or repress the expression of iron deficiency responses. Alternatively, the *frd3* mutant phenotype could result from incorrect localization of iron in the shoot. If the iron in the shoots was unavailable to cells or organelles that generate the iron deficiency signal, the roots would be appropriately responding to a need for additional iron in portions of the shoot. However, it is not straightforward to explain how the loss of a protein expressed in the roots results in alterations in iron localization in the shoot. Additional experiments are ongoing to distinguish between these two models and further characterize the functions of the FRD3 protein. A functional FRD3-GFP fusion protein is only expressed in a subset of root vascular cells. FRD3 is being expressed in yeast in order to identify its substrate(s).

8-09 Identification and characterization of light stress sensitive Arabidopsis mutants

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Absorbed light energy which cannot be used for photosynthesis is one of the main sources for the formation of reactive oxygen species in chloroplasts and is an extremely harmful stress factor for photosynthetic organisms. Higher plants have developed a number of strategies to avoid or reduce damage which is related to these photo-oxidative stress conditions. To understand the entire response of the plant to photo-oxidative stress, light stress sensitive mutants from *Arabidopsis thaliana* identified by video imaging of chlorophyll fluorescence were isolated and characterized. The detailed analysis of mutants that are defective in the energy-dependent dissipation (qE-mechanism) of excess light energy underlined the importance of qE for the light-dependent down-regulation of photosystem II (PSII). However, this function of qE was not related to processes involving damage and repair of PSII. The protective role of qE was more pronounced at moderate light stress conditions and became less important with increasing light intensities indicating a role of qE in reducing the electron pressure on the photosynthetic electron transport chain. Studies of plant development under different light regimes further showed that qE is not required for the adaptation of plants to varying light intensities. Additionally, a number of light stress sensitive mutants with an unchanged capacity of thermal energy dissipation have been isolated. The screening procedure and the physiological characterization of these mutants will be presented.

8-10 The role of minor PSI subunits in the regulation of photosynthesis

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Plants in their natural environment are constantly exposed to changes in the light intensity and quality. In order to protect the photosynthetic apparatus against photoinhibition and optimise the photosynthetic performance, the activity of photosystem I (PSI) and photosystem II (PSII) has to be tightly regulated. We have analysed what role the minor PSI subunits plays in the regulation of PSI activity. Using sense and antisense techniques we have generated Arabidopsis thaliana plants lacking the nuclear encoded PSI-D, -E, -F, -G, -H, -K, -L or -N subunits. All these mutants are affected in their ability to fine-tune photosynthesis, but especially two subunits PSI-G and PSI-H play a central role in the regulation of PSI activity. Plants lacking PSI-G have a faster electron transfer from PSI to ferredoxin. We suggest that PSI-G regulates a shift between two working modes of PSI; a normal and a fast energy-quenching mode (Jensen et al., 2002). PSI-H, however, is important for the redistribution of a mobile light-harvesting complex (LHCII) between PSII and PSI. If PSI absorbs an insufficient amount of light energy, LHCII is redistributed from PSII to PSI. The redistribution is regulated by the redox state of the interchain electron carrier plastoquinone. In plants lacking PSI-H, however, LHCII remains bound to PSII even when plastoquinone is over-reduced (Lunde et al., 2000). Thus, the minor subunits of PSI are involved in several aspects of photosynthetic regulation.

8-11 *NEGATIVE HYDROTROPIC RESPONSE* gene is required for the perception of soil water through the *Arabidopsis thaliana* root tip

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In soil, plant roots constantly alter their growth direction in search of water. The root cap (RC) is not only the center for sensing physical and chemical stimuli but also for directing root growth orientation towards the stimuli. Hydrotropism of the root tips of young seedlings of *Arabidopsis thaliana* has been analyzed in a system with a created water potential gradient. In this system, wild type (wt) roots exhibit a tropic curvature and grow in the direction of the highest substrate water potential and against a gravitropic stimulus. We isolated a negative hydrotropic response (*nhr*) mutant that does not sense substrate water potential gradient and continued to grow into the media with the lowest water potential. *nhr1* seedlings had various anomalies in the RC morphogenesis including formative cell divisions. Physiological studies demonstrated that *nhr1* maybe impaired in auxin polar transport and abscisic acid pathways or in their interactions. *nhr1* roots developed significantly faster gravitropic responses to 90 degrees gravistimulations when growing on the surface of an agar medium. These data suggest that *nhr1* roots posses full gravitropic but negative hydrotropic competence. These results implied that *NEGATIVE HYDROTROPIC RESPONSE (NHR*) gene is required for *A. thaliana* roots to perceive soil water through the root cap and that hydrotropism is amenable to genetic analysis.

8-12 Ectopic expression of stress-inducible aldehyde dehydrogenase genes decreases oxidative stress and improves abiotic stress tolerance in *Arabidopsis thaliana*

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Formation of reactive oxygen species (ROS) is thought to be a major cause of cellular damage in plants as a result of dehydration, high salinity or heavy metal stress. Damage essentially arises from reactions of ROS with lipids and proteins resulting in the excessive accumulation of toxic degradation products. In an earlier study we have isolated novel ABA- and dehydration-inducible aldehyde dehydrogenases from the resurrection plant *Craterostigma plantagineum* (Cp-ALDH) and *Arabidopsis thaliana* (Ath-ALDH3) (Kirch et al., 2001. Plant J. 28, 555-567). Aldehyde dehydrogenases (ALDHs) belong to a large family of NAD(P)+-dependent enzymes that catalyze the irreversible oxidation of various aldehyde substrates. Kinetic data indicated that Cp-ALDH may be involved in the detoxification of reactive aldehyde species generated by oxidative stress-associated lipid peroxidation. Analysis of the transcript accumulation pattern shows that the *Arabidopsis* homologue *Ath-ALDH3* is upregulated after NaCl, heavy metal, H2O2 and paraquat treatments, suggesting a possible role in response to oxidative stress. To investigate the physiological role of the stress-responsive ALDHs we generated transgenic *Arabidopsis* plants constitutively overexpressing the *ALDH* cDNAs. Transgenic lines exhibit an improved tolerance in response to dehydration, salinity (NaCl), heavy metals (Cu2+ and Cd2+), paraquat and H2O2 under laboratory conditions. This tolerance is accompanied by a decreased accumulation of lipid peroxidation-derived reactive aldehydes (MDA) compared to wild type. The functional analysis of Cp-ALDH and Ath-ALDH3 in transgenic plants suggests that these proteins play an important role during osmotic stress response and participate in a novel oxidative stress protection and detoxification pathway.

8-13 *Arabidopsis thaliana* and *Arabidopsis halleri* as models: Molecular analysis of metal tolerance and metal signal transduction

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With the aim of identifying and characterizing molecular determinants of metal homeostasis, tolerance and hyperaccumulation we are using *Arabidopsis thaliana* and *Arabidopsis halleri* as model systems. *A. halleri* is a known Zn hyperaccumulator and also more Cd tolerant than *A. thaliana*. It grows, for instance, on sites in Central Europe contaminated with metals through medieval mining activities. We are mainly interested in (i) the contribution of known candiate genes to these differences and in (ii) elucidating regulatory processes within the plant metal homeostasis network. Using cDNA-AFLP a collection of metal-regulated *A. halleri* genes was established. Several putative signal transduction components have been studied extensively with regard to their regulation under different conditions in the two Arabidopsis species. A few of them, e.g. a transcription factor and a protein phosphatase 2C, are analyzed further using A. thaliana insertion lines. Metal responses are being analyzed for any changes caused by inactivation of a particular gene at the transcriptional level using microarrays and at the metabolite level using LC-ESI-Q-TOF-MS. A transcription factor knock-out line, for example, shows enhanced sensitivity towards Cu and Cd toxicity in the leaves, indicating a role of this protein in the expression of heavy metal and/ or oxidative stress responses. At the moment we are trying to identify the biochemical responses and the genes affected in their regulation by the respective transcription factor.

8-14 Characterization of two putative DNA glycosylases from *Arabidopsis thaliana*

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All living organisms must face the task of maintaining their genome integrity, continuously challenged by spontaneous chemical changes and by genotoxic agents of environmental origin. Although our knowledge about DNA repair mechanisms is well advanced in microorganisms and mammals, it is still underdeveloped in plants. The genome sequence of the model plant *Arabidopsis thaliana* provides a powerful tool for the identification of evolutionary conserved as well as plant-specific DNA repair mechanisms. Isolating full-length cDNAs for the correct annotation of two genomic sequences, we have identified in *A. thaliana* two genes encoding proteins that define a new family of DNA repair enzymes. The proteins are 1987 and 1365 amino acids long, and both show a C-terminal domain (240 aa) with extensive sequence similarity to DNA glycosylases from the HhH-GPD superfamily, including a conserved lysine residue and the FCL region. Similar protein sequences are encoded in the genome of other plants but not in archaea, bacteria, fungi or animals. This suggests that they may represent a plant-specific family of DNA repair enzymes. Both genes are actively expressed in different plant tissues, including leaves, flowers, shoots and roots. Overexpression studies are underway in order to determine the substrate specificity of these proteins.

8-15 Translesion synthesis DNA polymerases in *Arabidopsis thaliana*

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Translesion synthesis is an important mechanism used by cells to tolerate the presence of non-repaired DNA damage and overcome replication blockage. Recent studies in microorganisms and mammalian cells suggest that the key participants in this process are specialized DNA polymerases able to copy damaged DNA templates with different degrees of accuracy. These enzymes define a new family of structurally-related DNA polymerases typified by *E. coli* Pol V (UmuC) and Pol IV (DinB), and *S. Cerevisiae* Pol h and Rev1. We have identified in *A. thaliana* three genes (*AtPOLK*, *AtPOLH* and *AtREV1*) encoding putative homologues of Pol IV, Pol and Rev1, respectively. *AtPOLK* encodes a protein of 672 aa and it is expressed in a wide range of plant tissues. Its transcript undergoes alternative splicing generating at least 3 different mRNAs. Overexpression of *AtPOLK* cDNA in *E. coli* cells causes a significant increase in their spontaneous frameshift mutation frequency. Thus, like its bacterial and mammalian counterparts, AtPOLK may play a role in the extension of mismatched base pairs during normal DNA replication. *AtPOLH* encodes a protein of 672 aa and it is expressed in aerial plant tissues, but not in roots. Overexpression of *AtPOLH* in bacteria protects against the cytotoxic and mutagenic effect of UV light. Finally, *AtREV1* transcript undergoes alternative splicing generating at least four different mRNAs. Overexpression studies are underway in order to characterize the enzymatic activities of all three proteins.

8-16 Hormonal and molecular characterisation of root system architecture changes induced by phosphate starvation in *Arabidopsis thaliana*

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Phosphorus bioavailability is one of the most challenging problem in crop nutrition and, even in fertile soils, is often a limiting factor. Although inorganic orthophosphate (Pi), the assimilated form of phosphorus, could be present in sufficient amount, the high phosphate-soil binding capacity leads to very low Pi concentration in soils. As a consequence, assimilation, storage and metabolism of Pi are highly regulated processes that directly affect plant development. However, plants have developed various adaptive strategies to increase phosphorus acquisition efficiency by numerous physiological, biochemical, molecular and morphological adaptations traits. Among these, modification of the root system architecture allows the exploration of a larger volume of soil. Although large insights have been made in biochemical knowledge of the response to P starvation, very little is known about the hormonal control and the molecular events underlying the morphological responses.To address these questions, we described a fine analysis of the root system architecture changes induced by P starvation either on hormonal mutants or on wild type plants grown on medium containing various phytohormones (effectors and inhibitors). This extensive study was systematically associated to a transcriptome analysis. RNA extracted from mutants and WT plants cultivated on the different mediums were extracted and probed on microarrays covering 1300 unselected genes. A model presenting the involvement of phytohormones on the root morphological parameters under P starvation and their effect at the molecular level will be presented.

8-17 What is wrong with *soz2*? - A genetic and physiological characterization of the ozone-sensitive *Arabidopsis thaliana* mutant *soz2*

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Under normal growth conditions, the recessive ozone-sensitive *Arabidopsis thaliana* mutant *soz2* exhibits a pale phenotype compared to wild-type. When exposed to ozone, *soz2* leaves became even paler and contained an increased level of the lipid peroxidation product malondialdehyde (MDA) compared to wild-type. These data suggest that *soz2* could suffer from oxidative stress. When wild-type and *soz2* were infected with virulent *Pseudomonas syringae*, bacterial growth was significantly higher in the mutant than in wild-type. In line with this higher susceptibility of *soz2* to virulent *P. syringae*, PR5 (pathogenesis-related protein 5) protein expression was significantly lower in *soz2* than in wild-type as revealed by Western blot analysis. When wild-type and *soz2* were exposed to high-light stress, potential activities of photosystems I and II decreased to a similar extent in both genotypes, indicating that the photosynthetic apparatus was not affected by the *soz2* mutation and that *soz2* was not more susceptible to oxidative stress generated by high light in the chloroplast. Confocal and multiphoton microscopy revealed that the paleness of *soz2* is due a lower cell density in the leaf palisade parenchyma. The *SOZ2* gene has been identified using a combined positional cloning and a transgenic complementation approach. However, the function of the protein encoded by *SOZ2* is not yet known, anyhow we hypothesize that it is required for normal post-embryonic leaf development. In summary, the *SOZ2* gene may be involved in leaf development and indirectly in responses to oxidative stress that is generated in the apoplast.

8-18 A calcineurin B-like calcium sensor protein essential for integration of plant responses to abiotic stresses

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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 c alcineurin B-like (CBL) calcium sensor proteins from *Arabidopsis* and identified a group of plant-specific serine-threonine protein kinases (CIPKs, CBL-interacting protein kinases) as targets of these sensor proteins. A detailed analysis of protein-protein interactions revealed a conserved 24 aa domain within the otherwise variable C-terminal region of these kinases as necessary and sufficient to mediate interaction with the CBL proteins. Comparative CBL-CIPK interaction studies suggest preferential complex formation as one of the mechanisms generating the temporal and spatial specificity of calcium signals within plant cells. Thus distinct combinations of different CBL/CIPK proteins can form a complex network that connects extracellular signals to defined cellular responses. Here we will present the detailed the phenotypical analysis of a CBL "knock out" mutant. The specific loss of this calcium sensor protein renders the plants hypersensitive to several abiotic stresses, such as drought, salt and heat. Expression studies revealed that, in these plants, the induction of stress-induced genes is severely impaired. We will discuss these findings in the context of the available expression and interaction data of the CBL-CIPK network as possible mechanisms contributing to the required cross-talk and specificity in stress signal responses.

8-19 Increased cysteine availability confers tolerance to heavy metals

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Based in earlier works that showed *Atcys-3A* gene, coding for the cytosolic isoform of the enzyme that catalizes cysteine biosynthesis, is regulated by heavy metal stress conditions we decided to deeply investigate this observation. Upon cadmium and arsenite treatments an induction of *Atcys-3A* gene expression, enzyme activity and cysteine and glutathione contents are.observed. Arabidopsis-transformed plants overexpressing *Atcys-3A* show an increase tolerance to both heavy metals, which correlates with higher levels of metal accumulations in leaves. We have also used nuclear microscopy techniques, Micro-PIXE, RBS and SEM, to investigate the site of metal localization within *Arabidopsis* leaves. When plants are grown in cadmium-rich environment, the metal is sequestered within the trichomes on the leaf surface, preferentelly in their central region. Transformed plants accumulate more cadmium in the trichomes than wild type plants. Our results suggest that increased cysteine availability is responsible for metal tolerance probably due to cysteine is required for the synthesis of phytochelatins necessary for metal detoxification. Thus using this genetic approach we are now developing plants, useful for phytoremediation of heavy-metal contaminated environments.

8-20 Mapping and characterization of *Arabidopsis* light and ozone sensitive mutant *rcd2*

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Ozone is a gaseous air pollutant that is converted into reactive oxygen species (ROS) in cell walls of the affected tissues. The production of ROS is self-propagating even after the end of the initial exposure. Application of ozone to plant tissues thus creates first an O3-dependent extracellular oxidative burst, followed by subsequent plant cell-dependent, actively regulated oxidative burst. Therefore O3-exposure is a good tool to study signaling cascades that involve extracellular ROS formation in regulation of gene expression and cell death in intact plants. Components of this signaling, and related processes, can be identified and studied by using Arabidopsis mutants that are deficient in components regulating lesion development, and are thus showing alterations in the magnitude of lesion formation. Here we describe an ozone and light sensitive mutant, *rcd2* (for *r*adical induced *cell death2*) which is similar to *rcd1* (Overmyer et al., Pl. Cell 12:1849-1862, 2000) since it also displays hyper-sensitivity to ozone, superoxide and avirulent pathogens, but not to hydrogen peroxide. Under standard growth conditions *rcd2* exhibits a reticulate pattern of chlorosis. Under low light conditions (<50 mmol m-2 s-1) *rcd2* is indestinguishable from Col-0 wild type plants. This photobleaching phenotype is reminiscent of the *ppt1*, phosphoenolpyruvate/phosphate translocator. However, genetic complementation analysis show that *rcd2* is not an allele of *ppt1*. Map based cloning of *rcd2* is underway and preliminary analyses position the mutation in chromosome two at 71±3 cM. In addition, ozone-induced gene expression and responses of antioxidant levels of *rcd2* compared with Col-0 wildtype will be discussed.

8-21 Changes in cytosolic Ca^{2+} concentration and gene expression in response to brief low temperature exposures

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In plants, one of the responses to low temperature is a rapid and transient increase in cytosolic calcium ion concentration, [Ca2+] cyt, which has been suggested to be part of the signal transduction pathway leading to downstream responses such as induced gene expression. In order to investigate whether the induction of the Arabidopsis low temperature-regulated gene *LTI78* could be correlated with changes in [Ca2+]cyt, an experimental set-up was established where treatment conditions could be altered and subsequent changes in both [Ca2+]cyt and *LTI78* expression could be determined. Arabidopsis seedlings producing the Ca2+-binding luminescent protein aequorin were exposed to different temperatures and conditions that resulted in different rates of temperature decrease. These treatments resulted in different patterns of the [Ca2+]cyt transients, as well as an induction of *LTI78*. Accumulation of *LTI78* mRNA was observed after a low temperature exposure as brief as 30 seconds. However, this accumulation could not be detected immediately after the low temperature exposure, instead the mRNA was found to accumulate during the post treatment period. A correlation between the pattern of the calcium response and the level of gene expression was found. In addition, reduction of the [Ca2+]cyt response, by pre-treating the seedlings with the Ca2+ channel blocker LaCl3, also led to a reduced level of *LTI78* mRNA accumulation. These results show that brief exposures to low temperature results in the onset of a signalling pathway that leads to the induction of gene expression and indicate the involvement of changes in $[Ca^{2+}]_{cyt}$ in this signalling pathway.

8-22 RCI2: A new *Arabidopsis* protein family involved in abiotic stress responses

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RCI2A and *RCI2B* are two *Arabidopsis* Rare-Cold-Inducible 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. The complete sequence of the Arabidopsis genome has revealed that *RCI2A* and *RCI2B* belong to a gene family composed by 8 putative genes. The deduced amino acid sequence showed that all proteins have two potential transmembrane regions highly conserved, four of them showing an extra hydrophilic C-terminal domain of about 20 residues. Expression analysis allowed to detect messanger RNAs of 6 members of the *RCI2* family and showed that 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 different living organisms ranging from bacteria to nematodes, suggesting a conserved and important role through evolution. The deletion of the yeast protein PMP3, having high sequence similarity to RCI2A, increases the plasma membrane potential and confers sensitivity to cytosolic cations such as Na+ (Navarre and Goffeau, 2000; EMBO J. 19:2515-24). Interestingly, the expression of RCI2A protein in yeast can substitute for the loss of PMP3, indicating a common role for both proteins. Similar analysis conducted in our laboratory showed that some of the members of the RCI2 protein family, but not all, are able to replace PMP3, suggesting that RCI2 members have different functions.

8-23 A novel cold-inducible gene from *Arabidopsis*, *RCI3*, encodes a peroxidase that constitutes a component for stress tolerance

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A cDNA from *Arabidopsis* corresponding to a new cold-inducible gene, *RCI3* (for *Rare Cold Inducible* gene 3), was isolated. Isoelectric focusing electrophoresis and staining of peroxidase activity demonstrated that *RCI3* encodes an active cationic peroxidase. RNA-blot analysis revealed that *RCI3* expression in response to low temperature is negatively regulated by light, as *RCI3* transcripts were exclusively detected in etiolated seedlings and roots of adult plants. *RCI3* expression was also induced in etiolated seedlings, but not in roots, exposed to dehydration, salt stress or ABA, indicating that it is subjected to a complex regulation through different signaling pathways. Analysis of transgenic plants containing *RCI3::GUS* fusions established that this regulation occurs at the transcriptional level during plant development, and that cold-induced *RCI3* expression in roots is mainly restricted to the endodermis. Plants overexpressing *RCI3* showed an increase in dehydration and salt tolerance, while antisense suppression of *RCI3* expression gave dehydration- and salt-sensitive phenotypes. These results indicate that RCI3 is involved in the tolerance to both stresses in *Arabidopsis*, and illustrate that manipulation of *RCI3* has a potential with regard to plant improvement of stress tolerance.

8-24 Important roles of drought- and cold- inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*

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Raffinose family oligosaccharides (RFO), raffinose and stachyose, accumulating during seed development are thought to play some role in desiccation tolerance of seeds. However, functions of RFO in drought tolerance of plants have not been elucidated. Sugar analysis showed that drought-, high-salinity- and cold-treated *Arabidopsis* plants accumulate a large amount of raffinose and galactinol, but not stachyose. This suggests that raffinose and galactinol are involved in the tolerance to drought, high-salinity and cold stresses. Galactinol synthase (GolS) catalyzes the first step in the biosynthesis of RFO from UDP-galactose. We identified three stress-responsive *GolS* genes (*AtGolS1, 2 and 3*) among 7 *Arabidopsis GolS* genes. Overexpression of *AtGolS2* in transgenic *Arabidopsis* caused an increase in endogenous galactinol and raffinose, and showed reduced transpiration from leaves to improve drought tolerance. Microarray analysis did not reveal any genes that are significantly upregulated or downregulated in the *AtGolS2*-overexpressing plants, which suggests that galactinol and raffinose do not function as signaling molecules but funtion as osmoprotectants.

8-25 Discovering of phosphate sensitive root architectural mutant

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Phosphorus bioavailability is one of the most challenging problem in crop nutrition and, even in fertile soils, is often a limiting factor. Although inorganic orthophosphate (Pi), the assimilated form of phosphorus, could be present in sufficient amount, the high phosphate-soil binding capacity leads to very low Pi concentration in soils. As a consequence, assimilation, storage and metabolism of Pi are highly regulated processes that directly affect plant development. Furthermore, plants have evolved various adaptive strategies to increase phosphorus acquisition efficiency including physiological, biochemical, molecular and morphological traits. Among these, modification of the root system architecture allows the exploration of a larger volume of soil. Although large insights have been made in biochemical knowledge of the response to P starvation, very little is known about the molecular events underlying the morphological responses. In this study, morphological characterisation and macroarray analysis of 155 transcription factors were combined to identify genes potentially related to the morphological changes triggered by phosphorus depletion. T-DNA mutant of the selected genes were analysed for root architecture phenotypes, leading to the discovery of two mutants lines; one harbouring insensitivity to phosphate deprivation, and one showing a shortened primary root but high lateral root growth under limiting phosphorus. The role of these genes will be discussed in terms of adaptative advantages under phosphorus starvation.

8-26 A semidominant mutation in *Arabidopsis thaliana* high-affinity phosphate transporter AtPT1

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Phosphorous is the least bioavailable nutrient to plants, mostly found in soil as phosphate at concentrations around the micromolar range. Plants have developed a series of physiological and developmental responses in order to search for and scavenge the little phosphate available. A formerly prepared EMS mutant population, derived from an *Arabidopsis thaliana* transgenic line harbouring a reporter gene specifically responsive to phosphate deprivation (*AtIPS1::GUS*), was screened for individuals that constitutively expressed the construct under phosphate-plenty conditions. We have isolated and characterized a mutant with a semidominant phenotype, displaying both variable expressivity and penetrance in a heterozygous plant. The mutation responsible for this phenotype is an in-frame point mutation, which maps to the ORF coding for the high affinity phosphate transporter protein AtPT1. This semidominant condition could be the outcome of the mutant protein interfering with the wild-type transporter, or with other proteins not yet identified. New experiments are under way to evaluate these possibilities, as well as a potential role of AtPT1 in phosphate sensing.

8-27 Antagonistic roles of MAP kinases and protein phosphatases 2C in stress response

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The hormone abscisic acid (ABA) acts as a central mediator in adaptive responses to various environmental stress. Two loci, ABI1 and ABI2 (for ABA-Insensitive) that regulate the sensitivity to ABA have been particularly well characterised genetically, biochemically and their roles in conditioning guard cell movements have been probed by means of electrophysiology and pharmacology. These loci encode homologous protein phosphatases 2C (PP2C), and genetic inference based on analyses of recessive mutations at these loci suggest that they are negative regulators of ABA signal transduction and that they also negatively regulate their own expression. Thus, these PP2Cs probably function in a resetting mechanism to prevent stress responses constitutively which might be detrimental to the organism. In budding and fission yeasts, and in mammals, PP2C are known to be direct negative regulators of stress-activated MAP kinases. We have obtained preliminary evidence suggesting that certain stress-activated MAP kinases in Arabidopsis may be direct targets of negative regulation by ABI1 and ABI2. In the yeast two-hybrid as well as in vitro binding assays, the N-terminal non-catalytic domains of these PP2C display high affinity binding of one particular MAP kinase. We are currently exploring the possibility that ABI1 and ABI2 specifically dephosphorylate these stress-activated MAP kinases as part of the resetting mechanism to re-initiate plant growth. We are also interested in further defining the physiological functions of these particular MAP kinase cascades in the context of ABA signalling and stress response.

8-28 Function and regulation of the vernalization-responsive gene *EARLI1*

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Vernalization, a prolonged exposure of plants to cold, accelerates flowering in *FRIGIDA*-containing, late flowering, ecotypes of Arabidopsis. A subtractive hybridization approach identified *EARLI1* as a novel vernalization-responsive gene (1). In addition to being stably activated by vernalization, *EARLI1* is transiently induced in response to cold shock and was recently shown to be controlled by photoperiod. *EARLI1*'s regulation is being reconstituted using *EARLI1*::GUS fusions in transgenic plants to determine the minimal gene fragment that responds to vernalization, cold shock, and photoperiod. Loss-of-function and gain-of-function approaches are being used to determine whether the absence or over-abundance, respectively, of *EARLI1* affects flowering time, cold acclimation, and freezing tolerance. Progress towards understanding *EARLI1*'s function and environmental regulation will be presented.

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(1): Wilkosz and Schläppi (2000) Plant Mol. Biol. 44: 777-787.

8-29 Characterisation of P-type ATPase heavy metal transporter genes in *Arabidopsis*

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Among the P-type ATPase super family, there are seven putative heavy metal transporters in Arabidopsis. Our study focuses on three of these: AtAHM2, AtAHM3 and AtAHM4. The sequences of these three transporters contain the expected motifs for heavy metal transport P-type ATPases. However, in addition, they have extended C-terminal sequences containing possible heavy metal-binding domains. To investigate the function of these three genes we are:

(1) constructing promoter-GUS fusions in transgenic plants to examine the tissue-specificity of expression. In addition, RT-PCR is being used to measure the effect of heavy metal exposure on expression.
(2) T-DNA knock-out mutants for each gene have been isolated and are being studied for heavy metal sensitivity or resistance, and heavy metal uptake and accumulation. In addition, we are constructing multiple mutant lines to test for function redundancy.
(3) cDNAs of these genes are being expressed *in E. coli* and yeast strains to measure the kinetics and specificity of metal uptake by these transporters.

8-30 Molecular and genetic analyses of *Arabidopsis* mutants that display delayed senescence symptoms

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Senescence is a final stage of development, leading to death of leaf cells. Senescence is now clearly regarded as a genetically programmed and evolutionally acquired developmental process. Although identification of genes that alter senescence has practical value and is helpful in revealing pathways that influence senescence, the genetic mechanisms of senescence have been very limited. We have undertaken a systematic genetic screening for mutants that exhibit delayed leaf senescence symptoms from various mutant pools. Several putative delayed senescence mutants were isolated from an EMS-mutagenized pool. One of isolated mutants, *e6-4*, exhibited delayed senescence symptoms in age-dependent *in planta* leaf senescence as well as in dark-, methyl jasmonate-, ABA- and ethylene-induced senescence. The identification of the mutated genes is underway and will be reported soon. Other delayed leaf senescence mutants, *ore7* and *357-3* line, were isolated from activation tagging lines and promoter trap lines, respectively. In the case of *ore7*, leaf longevity was increased dramatically during age-dependent senescence as well as during hormone-induced senescence. Cloning of the *ORE7* revealed that this gene encodes a novel protein containing a sequence motif present in eukaryotic transcriptional regulators. Gus expression of the *357-3* line was induced in fully mature leaves and maintained in senescing leaves. This line also displayed delayed senescence phenotype, indicating that this approach is feasible in identifying senescence-associated genes as well as the effect of the knockout mutations in these genes. Other candidate lines from these pools also have been further studying.

8-31 *Bsi-1*, a salt-stress inducible gene in *Brassica napus*, shows osmotic and oxidative stress-dependent protein translocation in Arabidopsis

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Salt stress from environment is one of the most important factors that limit crop productivity. To study the molecular mechanisms of plant resposes to salt stress, four-day-old seedlings of *Brassica napus* were treated with 200 mM NaCl for 0, 0.5, 1, and 3 h and total RNA were extracted. Differential display analysis using these RNA as templates permitted identification of several partial cDNA clones that exhibited rapid gene expression patterns upon salt stress. Of these clones, the clone homologous with the tobacco harpin-inducible gene, *hin1*, was designated as *Bsi*. Two full length cDNA clones of *Bsi, Bsi-1 and Bsi-2*, were obtained from *B. napus* cDNA library. The transcript level of *Bsi-1* was also increased by the ethephon, hydrogen peroxide and methyl jasmonate treatments. Southern blot analysis indicate that *Bsi* consist of a small gene family in *B. napus*. To gain an insight into how Bsi-1 works in cellular level upon stress treatments, the subcellular localization of Bsi-1 was examined using Bsi-1-green fluorescent (GFP) fusion protein in Arabidopsis protoplast. Bsi-1 is mainly localized to endoplasmic reticulum. However, NaCl or hydrogen peroxide treatments induce Bsi-1-GFP translocation into cytosol. The results suggest that the osmotic and oxidative stress-dependent translocation of Bsi-1 is probably important for its function in the stress responses.

8-32 Characterization of *Arabidopsis thaliana* homologue of *Schizosaccharomyces pombe rad17* checkpoint gene

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Cell cycle checkpoint controls are gating mechanisms that govern cell cycle progression in the presence of DNA damage and incomplete DNA replication, thus ensuring genomic stability and integrity. Studies in fission yeast implicate members of the Rad family of checkpoint proteins, including Rad17, Rad1, Rad9 and Hus1, as key early-response elements during the activation of both the DNA damage and replication checkpoint pathways. On the basis of its predicted sequence similarity to *Schizosaccharomyces pombe* Rad17 (SpRad17), a cDNA encoding the first plant homologue of Rad17 (AtRad17) has now been isolated from *Arabidopsis thaliana*. The gene encodes a 599-amino-acid protein that is localized mainly in the nucleus. AtRad17 protein shares 23 and 27% amino acid sequence identity with SpRad17 and human Rad17 homologue, respectively, and exhibits significant homology especially in highly conserved replication factor-C-like domains including a "Walker A" motif. RNA blot analysis revealed that *Atrad17* gene is expressed ubiquitously in plant tissues at low levels but at relatively higher level in cultured cells. Disruption of the *Atrad17* by T-DNA resulted in increased sensitivity to methylmethane sulfonate. Telomere lengths of the mutant increased in successive generations whereas no difference in telomerase activity was detected in meristematic tissues between wild-type and the mutant plants, suggesting that AtRad17 functions as a negative regulator of the telomere length in contrast to SpRad17 as its positive regulator. Together, our data suggest that the evolutionarily conserved Rad17 is required for the DNA damage checkpoint control and telomere maintenance in plant cells.

8-33 Domain analysis of the DREB2A protein, a transcription factor that is involved in dehydration- and salt-stress response in *Arabidopsis*

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DREB1 and DREB2 are transcriptional factors that function in low-temperature- and drought-stress-responsive gene expression, respectively. These proteins specifically bind to DRE/CRT in the promoter region of stress-inducible genes. Three and two homologues exist for DREB1 and DREB2, respectively, and each DREB has a conserved ERF/AP2 DNA-binding domain. Overexpression of the DREB1 genes in transgenic Arabidopsis induces strong expression of their target stress inducible genes and results in improved stress tolerance to freezing, drought and high salinity. However, only weak expression of the target genes induced in the DREB2A overexpressing transgenic Arabidopsis. Therefore, the post-translational modification such as phosphorylation seems to be necessary for the activation of the DREB2A protein. To characterize activation mechanism of the DREB2A, we analyzed the transcriptional activation domain of DREB2A using Arabidopsis T87 cell protoplasts. The full-length DREB2A protein (amino acid1-335) increased expression of a GUS reporter gene driven by DRE 5 times higher than vector control. The C-terminal-deleted DREB2A that lacks the region between a.a. 253 and 335 no longer activated expression of the reporter gene. The region between a.a. 253 and 335 fused to the GAL4 binding-domain activated expression of the reporter gene. The transcriptional activation domain of DREB2A seems to exist in the region between a.a. 253 and 335. On the other hand, internal deletion between a.a. 135 and 165 of DREB2A increased expression of the reporter gene 6 times higher compared to the full length DREB2A protein. This region may play an important roll in the activation of DREB2A.

8-34 Overexpression of the beta-carotene hydroxylase gene in Arabidopsis leads to improved tolerance to high light and high temperature conditions

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Plant stress, caused by extreme environmental conditions, is already a major cause of yield reduction in crops. The threat of global environment change makes it increasingly important to generate crop plants that will withstand such conditions. Stress, particularly in high levels of sunlight, leads to the production of reactive oxygen species causing photo-oxidative cell damage. Carotenoids (present in the membranes of all photosynthetic organisms) help protect against such light-dependent oxidative damage. In plants, the xanthophyll cycle (the reversible interconversion of two particular carotenoids, violaxanthin and zeaxanthin) plays a key photoprotective role and is therefore a promising target for genetic engineering to enhance stress tolerance. Over-expression in *Arabidopsis thaliana* of the *chy*B gene encoding beta-carotene hydroxylase (converts beta-carotene to zeaxanthin) increased the xanthophyll cycle pool size two-fold whether grown under low (100 µmol photons m-2 sec-1) or moderate (400 µmol photons m-2 sec-1) light. This was not at the expense of other carotenoids as the total carotenoid content per unit chlorophyll *a* was increased whilst chlorophyll content remained unchanged. This extra xanthophyll was mostly associated with the photosystem II light-harvesting complexes (LHCII), which showed a 3-fold increase in the amount of bound xanthophyll. The resulting plants were more tolerant to high light and high temperature conditions, as evidenced by reduced leaf necrosis, reduced production of the stress indicator anthocyanin and reduced lipid peroxidation. Non-photochemcial quenching was unchanged in the plants and stress protection is likely to be due to the role of zeaxanthin in preventing oxidative damage of membranes.

8-35 Isolation and characterization of *Arabidopsis* mutants with deregulated *CBF2* expression

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The process of cold acclimation involves a high number of biochemical and physiological changes, most of them regulated at the gene expression level. The *CBF* genes (*CBF1-3*) from *Arabidopsis* are considered key regulatory genes that mediate cold-acclimation response and freezing tolerance. They are induced rapidly in response to low temperature, and encode transcriptional activators that bind to the CRT/DRE (CCGAC) regulatory element present in the promoters of some cold- and drought-inducible genes. Constitutive expression of *CBF* genes in transgenic *Arabidopsis* plants results in induced expression of those genes, and enhanced tolerance to freezing, drought and salt stress. How *CBF* gene expression is regulated in response to low temperature still constitutes an important question that remains to be elucidated. To further dissect the complexity of cold acclimation response and identify intermediates in *CBFs* signal transduction, we have isolated *Arabidopsis* mutants showing deregulated *CBF2* expression. This was achieved by introducing into *Arabidopsis* plants a chimeric gene consisting of the cold-responsive *CBF2* promoter fused to the coding sequence for the firefly luciferase (*LUC*) gene. EMS mutagenized M2 progeny with altered reporter activities were identified by LUC bioimaging followed by northern hybridization of the endogenous *CBF2* mRNA. We will report on the characterization of these mutants at the genetical, physiological and molecular level.

8-36 The gene family of peroxiredoxins in *Arabidopsis thaliana*: Localization, genetic regulation and function

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Peroxiredoxins (Prx) are heme-free peroxidases showing a broad substrate specifity as they reduce hydrogen peroxide, lipid peroxides and peroxynitrate. Based on sequence characteristics and their reaction mechanisms, they are grouped in four clans: 2-Cys Prx and Prx Q-like proteins which contain two catalytic cystein residues in distinct sequence enviroments, while 1-Cys PRX and type II Prx are characterised by one catalytic cystein. During peroxide detoxification, the catalytic Cys-residues are oxidized and need to be reduced by electron donors like thioredoxins, glutaredoxins or cyclophilins prior to the next catalytic cycle.In the *Arabidopsis* genome, 10 open reading frames were predicted to code for peroxiredoxins, which could be assigned to the four subgroups of Prx: two 2-Cys Prx, one 1-Cys Prx, one Prx Q and six Prx II. The Prx proteins show diverse subcellular localization in the nucleus, chloroplast, mitochondrium and probably in the cytosol. Expression analyses of the 7 prx genes expressed in green tissues revealed a highly variable and gene-specific regulation in response to reducing and oxidizing conditions. In addition, Prx proteins can be distinguished by their H2O2-reducing activity in vitro and their midpoint redox potential. The diverse subcellular localization, the specific redox-dependent regulation of gene expression and the biochemical characteristics give first insight into the particular function of each Prx. The results are discussed in terms of the physiological function of the Prx gene products.

8-37 ACTCAT is a *cis*-acting element involved in *Arabidopsis ProDH* gene expression by hypoosmolality 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* (*ß-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*. Microarray and RNA gel blot analyses showed that 21 L-Pro-inducible genes have the ACTCAT sequences in their promoter regions. These results indicate that the ACTCAT sequence plays an important role in the L-Pro-responsive gene expression. To isolate cDNAs encoding the ACTCAT sequence binding proteins we are now screening an *Arabidopsis* cDNA library using yeast one hybrid system.

8-38 Cation diffusion facilitator (CDF) proteins of *Arabidopsis*: A role in metal homeostasis?

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Essential metal cations like those of Cu, Fe, Mn, Ni or Zn are required as micronutrients. Since these transition metal cations display high affinities for organic ligands, and since some of them are able to participate in single-electron transfer redox reactions, availability inside the plant has to be tightly controlled in a metal homeostasis network involving uptake, sequestration, movement, trafficking and storage of these metals. In a functional genomics approach we are addressing the role in metal homeostasis of 13 Arabidopsis membrane proteins with some homology to *Saccharomyces cerevisiae* Zrc1p, *Ralstonia eutropha* CzcD, mammalian ZnTs and AtZAT of the cation diffusion facilitator family (Van der Zaal *et al., Plant Physiol*. 119, 1047-1055, 1999).To functionally characterize these proteins, heterologous expression was performed in a zinc-hypersensitive mutant of *S. cerevisiae*. Analysis of transcript levels by real-time PCR revealed differential expression in plant organs. In roots and shoots transcript levels responded to altered supply of transition metals. Transient expression of GFP fusion proteins is in progress in order to determine the subcellular localization of the proteins *in planta*. A number of T-DNA knockout lines have been obtained.

8-39 Arabidopsis leaf slices for biochemical and genetic effector studies

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Redox regulation of metabolic reactions and gene expression is a common principle in adjusting photosynthetically active cells to changing environmental conditions, for example to balance light absorption to energy consumption. Various levels of redox controlled adaptations can be distinguished, and range from fast metabolic responses such as state transition to slow changes of structure which involve alterations in gene expression. Two specific cDNA arrays were developed to quantify the expression levels of nuclear and plastid genes involved in redox regulation. One array contains candidate genes encoding antioxidative and redox-associated enzymes, redox modulators and stress inducible proteins. The second array comprises all chloroplast encoded genes. The sets of genes were amplified via RT-PCR and spotted on nylon membranes. The sensitivity and reliability of the experimental system was evaluated and a procedure was developed for detecting differential gene expression.To test the effect of redox active reagents, a system was developed which allows fast and reliable application to leaves. Incubation of infiltrated leaf slices in the presence of different effectors proved to be a suitable experimental approach. Gene expression profiles in response to redox active effectors will be discussed in context with the adjustment of photosynthetic cells to the prevailing environmental conditions.

8-40 Towards the characterisation of heavy metal tolerance in *Thlaspi caerulescens*

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Metal hyperaccumulator plant species constitute ideal model systems to study metal accumulation and tolerance mechanisms. Except few examples concerning metal uptake systems and synthesis of metal chelators, the molecular basis of metal homeostasis is still under documented. Strategies based on yeast complementation by plant cDNA libraries to identify genes involved in resistance to various abiotic stresses have already been described. The probability to find a particular gene is influenced, however, by the starting material, e.g. : desiccated organs to search for osmotic stress tolerance. We decided, therefore, to rely on biodiversity by choosing a plant naturally adapted to metalliferous soils which belongs to the *Brassicaceae* family and is phylogenetically close to *Arabidopsis thaliana: Thlaspi caerulescens*. Indeed, this plant is not only able to grow on toxic soils but also to accumulate high levels of metals in its tissues. These findings suggest the development of resistance mechanisms at the cellular level within these tissues. Our results indicate that *T. caerulescens* is not only able to accumulate nickel to high level in its aerial part when grown hydroponically but also exibits nickel resistance at the cellular level as demonstrated by protoplast survey assays. A cDNA library from *T. caerulescens* leaves was constructed in an expression vector and screened for its ability to render yeast cells tolerant against toxic nickel concentration. Isolated cDNAs will be presented and the involvment of their product in metal tolerance will be discussed.

8-41 Isolation and characterization of genes that are rapidly induced following a pulse of singlet oxygen in the conditional *flu* mutant of *A. thaliana*

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A strategy has been developed to study the role of singlet oxygen as a signal molecule in stress responses. The conditional *flu* mutant of *A. thaliana* releases a pulse of singlet oxygen within the first minute of illumination after a dark period. This precise timing *in planta* allows us to screen for genes which are rapidly induced following a singlet oxygen burst and therefore potentially act as downstream signal components. Seven genes with increased transcript levels after 20 minutes of illumination were identified by differential display. Six of the seven genes were induced transiently with a maximum transcript level between 30 to 60 minutes. The genes encode among others two zinc finger proteins, a receptor like kinase and a putative lipase. Gene activation, except for the lipase, occurred independently of salicylic acid in the *flu* mutant. Overexpression of a zinc finger protein in transgenic plants yields an early senescence-like phenotype with necrotic lesions and growth inhibition. The necrosis but not the growth inhibition was shown to be dependent on salicylic acid. The precise timing and rapid induction of early response genes in the *flu* mutant will be used to dissect the complexity of signaling events that lead to drastic stress responses following the initial release of singlet oxygen.

8-42 Transcriptome analysis of mineral nutrition

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Plants constantly have to adjust ion and water transport activities in response to an ever changing environment. Functional analysis of the complex network of cross interactions between transport activities must take into account the fact that the products of multiple genes and of even multigene families are responsible for the uptake and subsequent transport in the plant. This has been even more dramatically evidenced with the release of the complete *Arabidopsis* genome sequence. From that very moment, *in silico* analysis revealed an unexpectedly high proportion of transporter encoding genes orphan of any experimental evidence regarding their involvement in biological processes. As a matter of fact, this situation drives the design of gene specific targets (GSTs) in order to enable correct and complete molecular analysis of these transporters. A complete GST collection of about 200 *Arabidopsis* ionic transporters has been designed, and will be used in DNA-array expression studies in response to numerous aspects of physiological processes, where ionic fluxes are known to be of paramount importance. This study provides a standardised molecular basis for analysing the various cross interactions observed at the physiological level between distinct ionic transporter families. It also permits not only to better access the behaviour of genes previously implicated in a given process but also to identify still uncharacterized genes, thus facilitating the functional analysis of both wild type and mutant genomes. Current results obtained in response to salt stress will be presented.

8-43 Regulation of metal uptake in Arabidopsis

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Iron, an essential nutrient, is not readily available to plants due to its low solubility. In addition, iron is toxic in excess, catalyzing the formation of hydroxyl radicals. Consequently, plants must carefully regulate iron uptake. The Arabidopsis *IRT1* gene encodes the major transporter responsible for high affinity iron uptake from the soil. The steady-state level of *IRT1* mRNA is induced within 24 hours after transfer of plants to iron-deficient conditions, with protein levels peaking 72 hours after transfer. *IRT1* mRNA and protein are undetectable 12 hours after shifting plants back to iron-sufficient conditions. Overexpression of IRT1 did not confer dominant gain of function enhancement of metal uptake. Analysis of *35S-IRT1* transgenic plants revealed that although *IRT1* mRNA is constitutively expressed in these plants, IRT1 protein is only present in the roots when iron is limiting. Under these conditions, plants over-expressing IRT1 accumulate higher levels of cadmium and zinc than wild type plants, indicating that IRT1 is responsible for uptake of these metals and that IRT1 protein levels are indeed elevated in these plants. Our results suggest that expression of IRT1 is controlled by two distinct mechanisms that provide an effective means for regulating metal transport in response to changing environmental conditions. *35S-IRT1* plants show enhanced sensitivity to cadmium under iron-deficiency conditions. 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.

8-44 Analysis of genes encoding possible nickel-ion transporters in plants

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An understanding of metal transport in plants is important because of the role of certain trace elements as micronutrients in plants and because metal-tolerant plants might be useful in the phytoremediation of metal-contaminated land. While considerable progress has been made in understanding the molecular basis of iron, zinc and cadmium transport in plants, very little is known about the mechanisms involved in the transport of nickel, which in plants is found as an essential component of the enzyme urease. In microorganisms, nickel- and cobalt-specific transporters have been identified in the NiCoT permease family (2.A.52 in the transport protein classification). Two genes (AY049256 and AC005825) with similarity to NiCoT family have been identified in the *Arabidopsis thaliana* genome. To investigate the function of these genes in plants, we are investigating their expression characteristics in *Arabidopsis* and have cloned homologous cDNAs from the nickel-hyperaccumulator plant *Alyssum lesbiacum*. Hydropathy analysis predicts that these gene products have six transmembrane spans, and a possible transport function is being studied in heterologous expression systems. Investigation of the expression of these genes *in planta* is being conducted by northern analysis and the study of promoter-GUS fusions, which suggest that these genes may be highly expressed in the vasculature and trichomes. T-DNA knock-out lines have also been identified that will help to define the function of members of this gene family in plant growth and development and their possible involvement in metal-ion homeostasis.

8-45 Copper delivery to the secretory pathway in *Arabidopsis thaliana*

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Several *Arabidopsis* cuproproteins (i.e. the ethylene receptor or ascorbate oxidase) incorporate copper at the secretory pathway. At least three *Arabidopsis* homeostasis factors may contribute to provide copper to those proteins: COPT1, a high affinity plasma membrane copper transporter; CCH, a soluble cytosolic metallochaperone and RAN1, a P-type Cu-transporting ATPase of internal membranes. In this work, new putative components of this pathway are identified by sequence homology searches in the *Arabidopsis* genome. A five members family of COPT-related genes is described and the expression pattern of *COPT1* is analyzed by the generation of *COPT1::uidA* transgenic plants. GUS activity localizes at root tips, stomata, trichomes, and pollen. Moreover, *CaMV35S::COPT1* antisense transgenic plants exhibit alterations in pollen morphology, but unexpectedly ethylene perception remains unaltered. *CCH* antisense plants also display a normal ethylene response. The metallochaperone CCH, in addition to the conserved N-domain responsible for copper binding, contains a plant-exclusive C-domain of unknown function. A predicted gene product lacking this C-domain has been found in the *Arabidopsis* genome. Finally, five P-type ATPases with homology to RAN1 are also identified. One of them, temptatively denominated RAN2, has been chosen for further analysis based on its high similarity to RAN1. Interactions among metallochaperones and P-type ATPases transporters have been studied by the two-hybrid technique. Both RAN1 and RAN2 N-domains interact with the CCH homolog but not with CCH under the conditions tested. A model based on targeting signals prediction and the obtained results is discussed in the context of copper in *Arabidopsis thaliana.*

8-46 An activation-tagging mutation that reduces the vernalization response

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Winter annual ecotypes of *Arabidopsis* are late flowering unless exposed to a long period of low temperature (known as vernalization). We have found a mutation that renders winter-annual types of *Arabidopsis* insensitive to vernalization. The mutation resulted from an insertion of activation-tag vector, pSKI015. Identification of T-DNA flanking sequence and constitutive expression of a candidate gene revealed that mutation is caused by increased expression of an ORF which does not show similarity to any other known genes. This gene has a highly conserved relative in *Arabidopsis* and constitutive expression of that also causes similar vernalization insensitive phenotype, implying functional redundancy. RNA levels of *FLC* are not decreased by vernalization in this activation-tagged mutant. Moreover, RNA levels of these cloned genes are also regulated by vernalization, suggesting the possible role of these genes in the vernalization pathway.

8-47 Differential regulation of the L3 ribosomal protein in Arabidopsis

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Ribosomes are essential organelles composed of ribosomal RNA (rRNA) and ribosomal proteins (rProteins). Stochiometric amounts of each rProtein must be present for efficient assembly of ribosomes. In *E. coli* each rProtein is encoded by a single gene. In mammals there are small gene families for each rProtein, however it appears that only one member is functional. In *Arabidopsis*, small gene families with two or three functional members, encode most proteins. The deduced amino acid sequence of each member in plants varies from identical to 74% identity. We have characterized two members of a ribosomal protein gene family (L3A and L3B) from *Arabidopsis*. These two genes share approximately 86% amino acid identity. Based on Western analysis, the L3A protein appears to be essentially constitutively expressed at high levels in all tissues. L3B is more abundant in root than shoot. L3B mRNA has several unusual features. Its 5' UTR is very short, but is otherwise normal and, it has a very short polyA tail. We have developed some evidence that mRNA processing may be involved in the regulation of L3B levels. A T-DNA insertion mutant from the SALK collection has been identified for L3A. The mutation appears to lethal when homozygous, and the hemizygote shows slower growth compared to wild type. In addition, the insertion is transmitted to the F2 generation from hemizygote parents in 1:1 ratios instead of the expected 2:1.

8-48 Ancient classes of plant metallothioneins bind nutrient and toxic metals differentially

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Metallothioneins (MTs) are small proteins, typically less than 85 amino acids, which appear to be ubiquitous in eukaryotes and cyanobacteria. They have two to three cysteine-rich metal binding domains separated by a linker region. We have shown the existence of four ancient classes of MTs in *Arabidopsis* based on distinct cysteine spacing patterns in MT metal binding domains. Each class contains MTs from a variety of plant species, including wheat, rice and pine. All four ancient classes of MTs predate the monocot-dicot divergence that occurred 200 million years ago. These ancient classes of MTs may have been preserved in the plant genome because their distinct metal binding properties provide protection from toxic metals and elevated levels of nutrient metals. We have examined MT protein stability *in vivo* and determined differential MT metal affinities for each class. Contrary to current dogma, distinct *in vivo* metal binding affinities were found among representatives of three ancient MT classes that do not correlate with the *in vitro* thiol reactivity of several divalent metal ions tested. Additionally, we have exploited the extreme divergence between MT classes in an internal linker region to generate class-specific monoclonal antibodies for immunohistochemical experiments. These antibodies will be used to determine the distribution of MTs in plants at the organ, tissue and subcellular levels, which may provide additional fundamental clues about MT functions *in vivo*.

8-49 Iron uptake in Arabidopsis

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Iron deficiency is the most prevalent human nutritional disorder. In addition, plant growth is often limited by iron availability. Since most people obtain their dietary iron from plants, understanding how plants aquire iron may lead to improvements in human nutrition as well as improvements in crop yield. Our lab is identifying components involved in iron uptake to better understand this process in Arabidopsis. In response to iron deprivation, Arabidopsis induce both ferric chelate reductase activity and ferrous iron transport activity. We have now identified genes that encode the ferric chelate reductase (*FRO2*) and the ferrous iron transporter (*IRT1*) in Arabidopsis. We have also isolated a mutant, *frd3*, which demonstrates constitutive ferric chelate reductase activity. The transcripts of *FRO2* and *IRT1* are misregulated in *frd3*, accumulating under both iron deficient and sufficient conditions. Using a microarray experiment, we have identified a transcription factor misregulated in *frd3* named FIT1 (Ferrous-deficency Induced Transcripton Factor). FIT1 is a group B basic helix-loop-helix transcription factor. The predicted DNA binding sequence of FIT1 is located in the promoters of *IRT1* and *FRO2* indicating that FIT1 may directly induce transcription of these iron uptake genes. *FIT1* shows induced expression in iron deficient roots. In situ hybridization has shown *FIT1* mRNA to be present in iron deficient root epidermal cells. We are overexpressing *FIT1 in planta* and are isolating a T-DNA insertion line to study the effect of *FIT1* mRNA abundance on the expression of ZIP family members.

8-50 Discrimination of cold and drought signals by the AP2 transcription factors DREB1 and DREB2

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Low temperature and drought environmental stresses induce very similar sets of genes in Arabidopsis that are thought to play a role in protecting cells against stress-induced damage. DREB proteins are transcription factors that bind to the DRE promoter element of such genes (e.g. *RD29A*). These were isolated from a library of ABA-independent transcripts by a yeast one-hybrid screen using 71bp concatemer of *RD29A* promoter DRE elements. The screen pulled out two AP2 transcription factors: DREB1A and DREB2A. It was shown that both DREB1 and DREB2 are small gene families and most significantly DREB1 mRNAs are induced by cold and those of DREB2 are induced by drought stress (Liu et al., Plant Cell 10: 1391-1406). Therefore, these transcription factors play a key role in discrimination of cold and drought. In order to identify the promoter elements of DREB1 and DREB2 regulated by cold or drought specifically, we have generated transgenic Arabidopsis plants that express luciferase under the control of 100 bp deletion series of these promoters. Interpretation of the luminescence imaging data obtained from these transgenic lines in response to cold and drought will be discussed. The transcription regulation of DREB2 in the wild type and in ABA signalling mutants in response to abiotic stresses other than drought will also be presented.

8-51 Analysis of transgenic plants overexpressing dominant-negative ATHK1 cDNAs in Arabidopsis

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We have cloned a cDNA encoding a hybrid-type histidine kinase ATHK1 from Arabidopsis. We then demonstrated that ATHK1 has the potential ability to act as an osmosensor by analyzing both 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 screened dominant-negative ATHK1 mutants that inhibit 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 ATHK1-6 has a nucleotide substitution at a putative ATP binding site. We then generated transgenic Arabidopsis plants overexpressing the dominant-negative ATHK1 cDNAs, and found that several lines exhibit late germination, growth retardation, short roots, accumulation of anthocyanin and stomatal closure under the normal growth condition. cDNA microarry and following northern blot analyses indicated that a number of stress-inducible genes are constitutively expressed in the dominant-negative ATHK1 overexpressors under the unstressed condition. Moreover, the dominant-negative ATHK1 overexpressors were tolerant to dehydration and high salinity stresses than wild-type plants. These results, together with yeast genetic analysis, suggest that ATHK1 is an osmosensor in Arabidopsis.

8-52 Gene expression profiling analysis of wild-type and Arabidopsis mutants under chilling treatment

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Chilling is a common abiotic stress to plants which leads to significant economic loss in agriculture. To understand plant responses to chilling, we have profiled the transcriptome of Arabidopsis wild-type plants and 12 chilling-sensitive mutants under normal (22ºC) and chilling conditions (13ºC) using Arabidopsis GeneChipTM microarrays. The expression of approximately 20% of the genes surveyed in the wild-type was significantly affected by exposure to the chilling temperature. The expression of genes encoding proteins involved in multiple biochemical processes such as protein synthesis and lipid metabolism was regulated by the low temperature treatment. The expression of more than half of these genes was also affected in the chilling-sensitive mutants. Results from cluster analyses performed to organize mutants into subclasses according to their molecular phenotype were consistent with previous classifications based on their visible phenotypes and on their complementation groups. Furthermore, the number of genes and the genes in different pathways whose expression was significantly altered by chilling reflected the severity of the chilling phenotype in each class. A comparison of the effects on gene expression caused by chilling temperature (13ºC) and cold stress (4ºC) will also be discussed.

8-53 Mutation in the *Abf1* gene, encoding a transcriptional regulator for ABA responsive genes, affects cold acclimation in Arabidopsis

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The phytohormone abscisic acid (ABA) regulates several different processes in plants including seed maturation and germination as well as vegetative responses to different environmental stresses. Low temperature is a severe environmental stress and plants are responding to low temperature partly through ABA signaling resulting in cold acclimation, by which plants can increase their freezing tolerance. Application of ABA to the plants leads to increased freezing tolerance without any low temperature treatment and defects in ABA biosynthesis or responsiveness impair cold acclimation and development of freezing tolerance in Arabidopsis. Cold acclimation is correlated with specific gene expression and most of the low temperature induced genes are also responsive to ABA.Mutant analysis has defined several components involved in ABA signal transduction, some of which are acting during seed development and some during vegetative responses. In Arabidopsis the *Abf1* gene encodes a transcription factor presumably mediating ABA responses during environmental stress. We have been utilizing an *abf1* mutant of Arabidopsis and studied the cold acclimation process in this background. The *abf1* mutant was able to cold acclimate and increase its freezing tolerance approximately to the same level as the corresponding wild type. However, the acclimation was slower in the *abf1* mutant and clear differences in freezing tolerance were detected during the first days of acclimation. The effect of the *abf1* mutation in low temperature and ABA responsive gene expression during cold acclimation will be discussed.

8-54 The transcriptional response of Arabidopsis to genotoxic stress - a high density colony array study

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DNA repair is a universal mechanism to defend organisms against threats on their genetic material. DNA repair is well studied in prokaryotes, yeast and human, however, only little is known in plants. To understand more about DNA repair in plants we have applied high density colony arrays (HDCA) to characterize the transcriptional response of *Arabidopsis* to genotoxic stress. Due to the low abundance of the transcripts of repair genes, conditions of severe genotoxic stress (bleomycin plus mitomycin C) were applied to treat *Arabidopsis* cells. From mRNA isolated under induced conditions a cDNA library was constructed and transferred into *E. coli*. Transformants of this library were arrayed onto nylon membranes and resulted in HDCA. The same array was hybridised with probes isolated under induced or non-induced conditions, successively. By analysing 25 000 clones (covering more than 10 000 individual genes), about 30 genes showed significant induction. Among them the induction of the repair-relative gene *RNR2* (ribonucleotide reductase small subunit) and the gene encoding an AAA-type ATPase was found to be most pronounced (10-fold). Stress-responsive genes like *ER6* (ethylene responsive protein) and *ACP1* (NaCl-induced calcium-binding protein) also show an increase in transcript level. In addition, induction/repression of a row of genes without any obvious classification in overall homology searches was detected. Detailed sequence and motif analysis of these genes is in progress and will provide new information about factors involved in DNA repair in plants. The results indicate that transcriptome analysis with HDCA is a valuable method for a global DNA repair study in *Arabidopsis*.

8-55 PP2C phosphatase from *Medicago sativa* is inactivating and dephosphorylating MAPK in plant cells

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Protein phosphatases of type 2C (PP2Cs) play important roles in regulation of eukaryotic signal transduction. PP2Cs act as monomer proteins, in contrast to protein phosphatases of type 1, 2A and 2B, indicating that this class of phosphatases contains all the necessary information for substrate specificity and regulation. In plants, a number of PP2Cs are known, but significant similarity is found only within a conserved phosphatase domain with different N-terminal extensions of variable length. We have isolated an alfalfa MP2C (1) and shown that a wound-induced MP2C, is a negative regulator of mitogen-activated protein kinase (MAPK) pathways. The N-terminus of MP2C contains a consensus MAPK docking motif that is conserved in several mammalian MAPK-interacting proteins. Structure-function analysis of MP2C and two other plant PP2Cs, ABI2 and AtP2C-HA, revealed that the N-terminus is involved in determining the substrate specificity of the phosphatases. In this report, we provide evidence that transiently expressed MAPK SIMK is activated by salt with the same kinetics as in planta and that MP2C, but not other PP2Cs inactivates MAPK in vivo when coexpressed transiently in protoplasts.

1.Meskiene, I., Bogre, L., Glaser, W., Balog, J., Brandstotter, M., Zwerger, K., Ammerer, G., Hirt, H. 1998. PNAS USA 95: 1938-43.

8-56 Molecular responses to abiotic stress in Canola during early stages of plant development

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Differential cDNA subtraction analysis from *Brassica napus* (14-day seedlings) grown under drought conditions was conducted. Over 143 clones were identified whose BLAST DNA sequence analysis revealed them to be constituents of 21 unique genes. Most of the clones displayed sequence homologies to previously characterized genes, with the exception of a few that showed no homology to any known sequences. These 21 genes were analyzed for their differential induction or suppression, by Northern blot analysis of total RNA from drought stressed and control plants. Northern blot analysis confirmed the response pattern of drought stress on the corresponding mRNAs, with the exception of two genes that showed no response or very weak induction. The effect of drought varied from dramatic to slight induction or reduction in the steady state of the corresponding mRNAs. Out of 19 genes, 13 were induced and 6 were suppressed by the drought treatment. Furthermore, while a few of these genes displayed a drought-specific response, the majority appeared responsive to other abiotic stresses such as low temperature and high salinity.

8-57 A mutational analysis of the *ABA1* gene in *Arabidopsis thaliana*

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The important roles played by the hormone abscisic acid (ABA) in plant responses to various environmental stresses are well known. In addition to these physiological phenomena, ABA is known to be involved in seed development and dormancy. The *ABA1* gene of *Arabidopsis thaliana* codes for the zeaxanthin epoxidase enzyme, which catalyzes the first two steps of the biosynthetic pathway of ABA. We describe here the molecular analysis of four previously isolated alleles (*aba1-1, aba1-3, aba1-4 and aba1-6)* and five novel alleles (*sañ1-1, sañ1-2, sañ1-3, sañ1-5 and sre3*) of the *ABA1* gene. The comparative study of the phenotypes of these *aba1* mutants makes it possible to discuss the mechanisms of zeaxanthin epoxidase catalysis and regulation as well as the putative existence of an alternative pathway for ABA synthesis. Some of the mutants studied here are likely to carry null *aba1* alleles and display morphological alterations suggesting the implication of ABA in vegetative developmental events.

8-58 Mapping QTL for salt tolerance

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The little success of breeding approaches to the improvement of salt tolerance in crop species is thought to be due to the quantitative nature of most, if not all the processes implicated. Hence, the identification of some of the quantitative trait loci (QTL) that contribute to natural variation in salt tolerance should be instrumental to eventually manipulate the perception of salinity and the corresponding responses. Aiming to analyse natural variability in salt tolerance, we have compared the ability of 102 wild-type races of *Arabidopsis thaliana* to germinate on 250 mM NaCl, finding a wide range of variation among them. Ecotypes displaying extremely different responses to NaCl were intercrossed and the phenotypes found in their F2 progenies suggested that natural variation in salt tolerance during germination was under polygenic controls. Genetic distances calculated on the basis of variations in repeat number at 22 microsatellites, were analysed in a group of either extremely salt tolerant or extremely salt sensitive ecotypes. We found that most, but not all ecotypes with similar responses to NaCl are phylogenetically related. Salt tolerance was also studied in 100 recombinant inbred lines derived from a cross between the Col-4 and L*er*-0 ecotypes. We detected 11 QTL harboring naturally occurring alleles that contribute to natural variation in salt tolerance in *Arabidopsis thaliana*, 6 at the germination and 5 at the vegetative growth stages, respectively. At least 5 of these QTL are likely to represent loci not yet described by its relationship with salt stress.

8-59 Molecular characterization of the *NHX* gene family of Na^+/H^+ antiporters in *Arabidopsis thaliana*

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The *Arabidopsis thaliana* vacuolar Na+/H+ antiporter AtNHX1 is a salt tolerance determinant. Five additional *AtNHX* genes (*AtNHX2*-6) have been identified and characterized. *AtNHX1* and 2 mRNAs are the most prevalent transcripts among this family of genes in seedling shoots and roots. A lower abundance *AtNHX5* mRNA is present in both shoots and roots whereas *AtNHX3* transcript is expressed predominatly in roots. *AtNHX4* and 6 mRNAs were detected only by RT-PCR. Seedling steady-state mRNA levels of *AtNHX1* and 2 increase similarly after treatment with NaCl, an equiosmolar concentration of sorbitol, or ABA, whereas *AtNHX5* transcript abundance increases only in response to salt treatment. Hyperosmotic upregulation of *AtNHX1*, 2 or 5 expression is not dependent on the SOS pathway that controls ion homeostasis, but transcription of these genes was negatively affected by the SOS pathway in absence of stress. *AtNHX1* and 2 transcripts accumulate in response to ABA but not to NaCl in the *aba2-1* mutant indicating that the osmotic responsiveness of these genes is ABA dependent. A yet undefined stress signal pathway that is ABA- and SOS-independent apparently controls transcriptional upregulation of *AtNHX5* expression by hypersaline shock. AtNHX1, 2 or 5 suppress, with differential efficacy, the Na+/Li+ sensitive phenotype of a yeast Δ*nhx1* mutant. Ion accumulation data indicate that these AtNHX proteins function to facilitate Na+ ion compartmentalization and maintain intracellular K+ status. Similar to AtNHX1, AtNHX2 is localized to the tonoplast of plant cells. Together, these results implicate AtNHX2 and 5, together with AtNHX1, as salt tolerance determinants.

8-60 Reconstitution of the Arabidopsis SOS signaling pathway for Na^+ homeostasis

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The *Arabidopsis thaliana* SOS1 protein is a Na+ transporter that is essential for the NaCl tolerance of plants. *sos1* mutant plants share phenotypic similarities with mutants lacking the protein kinase SOS2 and the Ca2+ sensor SOS3, including defective K+ uptake at low external concentrations. To investigate whether the three SOS proteins function in the same response pathway, we have reconstituted the SOS system in yeast cells. Expression of SOS1 improved the Na+ tolerance of yeast cells through the promotion of Na+ extrusion at the plasma membrane. Co-expression of SOS2 and SOS3 dramatically increased SOS1-dependent Na+ tolerance, whereas SOS2 or SOS3 individually had no effect. The SOS2/SOS3 kinase complex mediated the phosphorylation of SOS1. In addition to kinase activation, SOS3 functions to recruits SOS2 to the plasma membrane. Accordingly, a constitutively active form of SOS2 phosphorylated SOS1 *in vitro* independently of SOS3, but could not fully substitute for the SOS2/SOS3 kinase complex for activation of SOS1 *in vivo*. Neither the unmodified nor the SOS2/SOS3-activated SOS1 protein showed K+ transport capacity *in vivo*, suggesting that the function of SOS1 in K+ uptake is indirect. Our results are the first example of functional reconstitution of a plant response pathway in a heterologous system and demonstrate that the SOS1 ion transporter, the SOS2 protein kinase and its associated Ca2+ sensor SOS3, constitute a functional module.

8-61 The complex network of heat stress transcription factors in Arabidopsis - how many are needed?

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Heat stress transcription factors (Hsfs) are the terminal components of a signal transduction chain mediating the activation of genes responsive to heat stress in particular. Sequencing of the Arabidopsis thaliana genome revealed the existence of 21 ORFs encoding putative Hsfs. By structural characteristics and phylogenetic comparison, the 21 representatives are assigned to class A, B and C.The functional characterization give first insights into the complex network of Hsfs responsible for plant's response to heat stress: All 21 Hsfs are expressed at least on the transcript level. But the expression levels change in response to heat stress and/or developmental signals. Similar to other proteins regulating gene activity, Hsfs have a modular structure. We analyzed the DNA binding, oligomerization state, nuclear import and export as well as transcription activation potential of the 21 Hsfs.Essential for transcriptional activity of class A Hsfs are short peptide motifs enriched in aromatic and large hydrophobic amino acid residues embedded in an acidic surrounding (AHA motifs), which interact with components of the transcriptional machinery. The six class B and class C Hsfs have no activator function on their own, but may act as coregulators of class A Hsfs.

8-62 Gravitational effects on gene expression: Changes in the Arabidopsis transcriptome under g-stress

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Callus cultures of *Arabidopsis thaliana* (cv. columbia) in Petri dishes were exposed to altered g-forces by centrifugation (1 to 10g). Transcripts of the gene coding for the starch hydrolyzing enzyme, amylase, were used to monitor threshold conditions for g-number and time of exposure which led to altered amounts of the gene product. Exposure to approx. 7 g for 1h resulted in increased transcript levels. As this treatment also caused alterations of transcript levels of other gene products (not shown), we used this hypergravity treatment for a microarray analysis, using a commercial *A. thaliana* chip with 4,105 unique annotated clusters / genes (IncyteGenomics). Transcripts of more than 200 genes were significantly increased in amount (ratio 7g / 1g control; 21.6 and larger). They fall into several categories. Transcripts coding for enzymes of major pathways form the largest group (25%), followed by gene products involved in cellular organisation and cell wall formation / rearrangement (17%), signalling, phosphorylation/dephosphorylation (12%), proteolysis and transport (10% each), hormone synthesis plus related events (8%), defense (4%), stress-response (2%), and gravisensing (2%). Many of the alterations are part of a general stress response, but some changes related to the synthesis / rearrangement of cell wall components could be more hyper-g-specific. We only found few gene products which were decreased in relation to 1g controls, and these were less significant (ratio < 21.6). We thus assume that g-forces above a threshold of about 5g for 1h are sensed by plant cells in general, causing distinct metabolic responses which obviously in part are regulated by gene expression, and we hypothezise that similar cascades of responses could be induces by gravitational forces below 1.

8-63 Stress tolerance in Arabidopsis: mutants, genes and signaling pathways

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In Arabidopsis thaliana, the Salt Overly Sensitive 1 (SOS1), Salt Overly Sensitive 2 (SOS2), Salt Overly Sensitive 3 (SOS3) and Salt Overly Sensitive 4 (SOS4) genes are required for intracellular Na+ and K+ homeostasis. Mutations in these genes cause Na+ and K+ imbalance and render plants more sensitive toward growth inhibition by salt stress. SOS3 is a myristoylated calcium-binding protein that maybe a sensor for cytosolic calcium signals elicited by salt stress. SOS2 encodes a serine/threonine protein kinase. SOS2 physically interacts with and is activated by SOS3. Salt stress up-regulation of SOS1, which encodes a plasma membrane Na+/H+ antiporter, is partly under control of the SOS3/SOS2 pathway. SOS2 also directly activates the Na+/H+ exchanger activity of SOS1. SOS4 encodes a pyridoxal kinase important for the biosynthesis of pyridoxal-5-phosphate, which could regulates SOS1 activity because SOS1 contains a putative pyridoxal-5-phosphate binding motif in its C-terminal cytoplasmic tail. These SOS genes define a novel regulatory pathway important for the control of intracellular ion homeostasis and salt tolerance in plants.SOS2-SOS3 interaction is mediated through the regulatory domain of SOS2. We have delimited within the SOS2 regulatory domain, a 21 amino acid motif (designated as the FISL motif) that is both necessary and sufficient for binding to SOS3. On the SOS3 side, no discrete motif could be identified that is sufficient for mediating the interaction with SOS2. It appears that the central EF-hand as well as the N-terminal and C-terminal regions of SOS3 is required for binding to SOS2. Deletion of the FISL motif or a Thr^{168}-to-Asp mutation results in a constitutively active SOS2 that is independent of SOS3 or Ca^{+}. Expression of the constitutively active SOS2 mutants in yeast or Arabidopsis is partially sufficient for SOS1 activation and salt tolerance. In addition, recent progress on the characterization and cloning of several Arabidopsis mutations that affect osmotic stress and ABA regulated gene transcription will be presented.

8-64 Challenging plants and analysing phenotypes

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Gauntlets are established as sets of growth conditions making Arabidopsis genotypes split into distinct phenotypes. We apply graded nutrient or environmental challenges forcing plants to defined qualitative or quantitative visual phenotypes. The flexible cultivation of plants under gradients of growth conditions takes advantage of a set of growth chambers that control light and temperature. In a first approach, defined sets of metabolites and enzyme activities are analysed via routine protocols running on a semiautomatic analysis platform. Therefore assays for measuring metabolites (sugars, hexose phosphates, starch, proteins, amino acids) as well as enzyme activities have been transferred to the microplate format. We further adapt and develop enzyme assays to this format and transfer them to the liquid handling robot. In a complementary approach, a more detailed analysis is performed on selected material using metabolite and transcripts profiling methods. The project leads us to develop a knowledge database of plant responses to nutrient limitation and other environmental challenges. Ultimately, gauntlets are applied to genetic diversity and mutant collections to identify genes involved in the adaptation of the plant to its environment.

8-65 Molecular regulation of root system adaptation to drought stress

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Plant morphology is governed by a combination of pre-determined genetic programs and signaling pathways that respond to environmental cues. The result is that even genetically identical plants can have very distinct appearances when grown in different environments, with different numbers of organs that assume different shapes and locations. We are studying the effect of environmental factors on root system morphology in *Arabidopsis thaliana* var. Columbia (Col). The continuous formation of lateral roots in the root system is genetically determined but their number and location is highly plastic, responding to the availability of water and nutrients. By manipulating the water levels in the surroundings of the plant we have established environmental conditions that cause the root system to have a highly branched morphology (when water is plentiful) or a completely unbranched morphology (under mild drought conditions). Further studies showed that the different morphologies occur because lateral root development is delayed under drought conditions, causing lateral root primordia to accumulate in the root system. Although the mechanism of the developmental delay is unknown, we recently found that it involves the phytohormone abscisic acid (ABA). We have isolated two mutants in lateral root development (*lrd*) that form branched root systems even under drought conditions. In addition, we have determined that Landsberg Erecta, previously shown to have reduced ABA signaling, resembles the *lrd* mutants under drought conditions. Our current progress on dissecting the genetic mechanisms that govern root system morphology and its modulation by environmental signals will be presented.

8-66 Functional analysis of Athb-12, an ABA-inducible homeobox protein in Arabidopsis

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The *Athb-12* gene is a homeobox gene, and has a putative leucine zipper closely linked to the homeodomain. The *Athb-12* gene was shown to be induced by exogenous abscisic acid(ABA). To identify the function of Athb-12, primary screening for selecting knockout mutants of the Athb-12 gene among T-DNA insertional mutant pools (From the UWBC) was performed. From primary screening, we found two super pools which had insertional mutations at -360bp and -763bp regions from the transcriptional start site of the *Athb-12* gene. Secondary PCR screening revealed the 164th and 219th pools contain the mutant plant. Now tertiary screeing is progressing. As the Athb-12 has a putative leucine zipper motif involved in protein-protein interaction, we are screening salt-induced Arabidopsis cDNA library by yeast two-hybrid system. Athb-12 is thought to function as a transcription factor through interaction with other proteins. Function of Athb-12 will be studied by the phenotype of Athb-12-knockedout-plant will be examined.

8-67 Analysis of phosphate signal transduction pathway

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Phosphate availability is considered as one of the major limiting factors for plant development. Plants respond to the level of soluble phosphate by activating specific mechanisms in an effort to increase phosphate uptake and utilization. This includes changes in root architecture and gene induction like high affinity phosphate transporter. An Arabidopsis thaliana line was selected in the T-DNA insertion mutants collection generated by the INRA station of Versailles. In this line the T-DNA interrupt the coding sequence of a high affinity phosphate transporter. The GUS gene trap inserted in this construct is functional and allows a direct spatial and temporal analysis of the transporter transcription. This gene is strongly up regulated during phosphate deficiency in the root apex and in two layers of differentiated part of this organ (cortex and epidermis). Physiological analysis demonstrated that in phosphate starvation conditions the induction of this gene increases the potential root absorption for this ion from 60 %. Such defect also promotes a reduction of root growth from nearly 50%. The transporter appears highly regulated by the amount of phosphate available and appears to be a good candidate for reporting deficiency of the phosphate signal transduction pathway. The line was therefore mutagenised (EMS treatment) and the progeny was screened for mutants exhibiting a deregulation of the transcription pattern. Numerous candidates have beenidentified in various conditions of phosphate supply suggesting the existence of a complex network of positive and negative regulation.

8-68 A mutation in a gene encoding a Na^+-Pi transporter-like protein mediates ion sensitivity of *Arabidopsis* seedlings

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Mutations (87) that cause salt sensitivity were identified in a screen of 94,000 T-DNA lines (C24 *RD29A::LUC*) based on growth inhibition of T2 seedlings after transfer to agar medium supplemented with 140 to 180 mM NaCl. One recessive mutation, *npct1* (Na+-dependent phosphate co-transporter), was found to be linked with ionic stress sensitivity. Root growth of *npct1* seedlings is less inhibited by NaCl than that of *sos1* (C24 background) seedlings. Shoot and root morphological growth patterns in response to salt stress are also different from *sos1*. On medium supplemented with 160 mM NaCl, *npct1* seedling root growth inhibition is characterized by swelling of the root tip and hairs and by increased lateral branching. Root growth and morphology of *npct1* seedlings is similar to C24 seedlings when grown without stress. Shoot growth and morphology of C24 and *npct1* seedlings appeared the same with or without stress. *npct1* seedlings are not hyperosmotic sensitive but exhibit differential ion sensitivities, Cl- > Na+ = K+. *AtNPCT1* expression (steady-state transcript level) is down regulated substantially by a T-DNA insertion that is located 680 bp upstream of the translation start site. AtNPCT1 has greatest sequence similarity to DNPI (differentiation-associated Na+/Pi transporter)/VGLUT2 (vesicular glutamate transporter) in animals. DNPI/VGLUT2 was identified initially as a plasma membrane Na+-dependent Pi transporter but is now known to mediate H+-dependent glutamate uptake into synaptic vesicles, a process that requires Cl-.

8-69 Investigation of *Arabidopsis thaliana Tps1* by functional expression in yeast

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The biosynthesis of trehalose has been studied extensively in yeast. Two enzymes, Tps1 and Tps2 are required to produce trehalose starting from UDP-glucose and glucose-6-P. In the first step, catalysed by the Tps1 enzyme, trehalose-6-P is formed wich is subsequently dephosporylated by the Tps2 enzyme into trehalose. A third subunit in the trehalose synthase complex, encoded by *TPS3* and *TSL1*, has a regulatory function. In yeast, trehalose plays an important role in resistance against heat, osmostress and droughtstress. Although trehalose can be found in many organisms, it is undetectable in nearly all higher plants. Only in some special cases, such as in the desert plant, *Selaginella lepidophylla*, high levels of trehalose are present. However, in the systematic sequencing projects of different plant species, EST sequences with homology to *Saccharomyces cerevisiae TPS1* have been found and the *Selaginella* and *Arabidopsis TPS1* genes have been isolated. Compared to the yeast Tps1 protein, the plant Tps1 proteins have long N- and C-terminal extensions of which the function is unknown. Deletion of the N-terminal part of the *Selaginella* and *Arabidopsis* Tps1 proteins strongly increases their activity after expression in yeast. Both TPS activity in cell extracts and trehalose accumulation in vivo are strongly enhanced. We have investigated the function of the N-terminal part of the *Arabidopsis* Tps1 protein with the use of inverse PCR and PCR mutagenesis. Precise regions in the N-terminus that are important for the inhibitory function of the N-terminus on the catalytic activity are identified. With two-hybrid analysis we proved that there is no direct interaction between the N-terminus and the other part of the Tps1 protein.

To obtain a higher stress resistance in plants, many groups have overexpressed the yeast *TPS1* or the *E.coli OtsA* gene (Goddijn, O. J. M. et al., 1997, Holmström, K. O. et al., 1996, Romero, C. et al., 1997, Pilon-Smiths et al., 1998). These transgenic plants showed a better drought tolerance in spite of a small increase in trehalose content. They also displayed a different photosynthetic activity, sugar partitioning and morphological changes which seem to be related to interference with glucose sensing mechanisms. A link between trehalose metabolism and glucose sensing through *AtHXK* has been proposed by several research groups (Paul, M. et al., 2001, Muller, J. et al., 1999). In search for evidence hereabout, we measured the growth capacity on glucose medium, the HXK activity and the metabolites in a yeast *hxk1Dhxk2Dglk1D* and *hxk1Dhxk2Dglk1D tps1D* strain overexpressing *AtHXKI*. Furthermore, UV mutagenesis has been used to obtain mutants in *AtHXKI* that increase HXK activity.

9-01 Understanding the roles of the ZTL/ADO1 and ZTL-like proteins (LKP2/ADO2 and FKF1/ADO3) in the control of the circadian rhythms in Arabidopsis

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In plants, the circadian clock controls daily changes of photosynthetic activities, leaf movement, cell growth and gene expression. We and others have identified a novel class of proteins that are closely associated with the control of the circadian period in Arabidopsis. The ZTL/ADO1 and ZTL-like proteins (LKP2/ADO2 and FKF1/ADO3) contain a PAS-like LOV domain, an F-box domain and a six kelch repeats domain. Mutations at *ztl*/*ado1* alter a wide range of clock-controlled responses, affecting the periodicity of two essentially unrelated processes: *CCR2* and *CAB* gene expression and cotyledon movement. These observations argue strongly that ZTL/ADO1 does not simply affect output but either affects on input to the clock or is an integral component of the circadian oscillator itself. The fact that ZTL/ADO1 physically interacts *in vitro* with both cryptochrome 1 and phytochrome B and the arrhytmicity observed under red light for a null *ztl*/*ado1* allele are consistent with both of these interpretations. That this arrythmicity is not observed for *ztl*/*ado1* under blue and white light might reflect activity of LKP2/ADO2 and FKF1/ADO3. Characterisation of a deletion mutant exhibiting a late flowering phenotype and alteration of rhytmic expression of clock-controlled genes resulted in the identification of *fkf1*/*ado3*. Following a reverse genetic approach we have recently isolated a loss of function allele for the third member of the family *LKP2*/*ADO2*. Unexpectedly, the *lkp2*/*ado2* mutant is not affected in any of the clock-controlled processes analysed, possibly indicative of a redundant function. To understand the roles of this novel gene family we have generated double and triple mutant combinations using null alleles for the *ado1*, *ado2*, and *ado3* mutants. The phenotypic analysis of these mutants will be presented.

9-02 Light control of Arabidopsis development, a role of protein degradation

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We are interested in the cellular signaling process responsible for regulation of development by extracellular stimuli. We used the light control of Arabidopsis seedling development as an experimental model. During our dissection of the genetic network involved in light control of Arabidopsis development, eleven pleiotropic COP/DET/FUS loci have been identified and revealed to be responsible for mediating light control of Arabidopsis seedling developmental program switch. Among them, COP1 is the master repressor of photomorphogenic development and acts within the nucleus as an E3 ligase by directly targeting photomorphogenesis-promoting transcription factors degradation by the 26S proteasome in darkness. Light inactivates COP1 and causes a reduction in its nuclear abundance. Another gene, COP10, encodes a likely E2 component. While most remaining genes encode subunits of a highly conserved multi-subunit protein complex, the COP9 signalosome, which defines a novel regulator of the E3 ligases and promotes de-conjugation of NEDD8/RUB1 from the certain E3 ligase. Therefore this group of regulators define new cellular machinery in regulating cellular responses to external stimuli or stresses. Currently, we are applying both molecular genetics and genomic approaches to further analyze this novel cellular machinery conserved among all multicellular organisms. Through this combinatorial approach we hope to better understand signaling mechanisms in how extracellular signals regulate this machinery pathway and how this machinery controls the genome expression pattern in response to the environmental signals.

9-03 Crucial role of *TOC1* in the control of morphogenic and circadian responses in Arabidopsis

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In Arabidopsis, the molecular components and intracellular signaling pathways underlying circadian clock function remain to be conclusively elucidated. Here, we show that under constant white light, silencing of the timing of cab expression 1 (*TOC1*) gene shortened the period length of circadian expression whereas increments in *TOC1* gene dosage delayed the pace of the clock. In addition, silencing of *TOC1* gene caused arrhythmia in constant darkness and in various intensities of red light while in blue light, made the clock to run faster than in wild-type conditions. Our results show that *TOC1* RNAi lines displayed an important reduction in sensitivity to red and far-red light in the control of hypocotyl elongation while increments in *TOC1* gene dosage clearly enhanced light sensitivity. Furthermore, the red-light mediated induction of *CCA1* expression was decreased in *TOC1* RNAi plants indicating a role for TOC1 in phytochrome regulation of circadian gene expression. We conclude that TOC1 is an important component of the circadian clock in Arabidopsis with a crucial function in the integration of light signals to control circadian and morphogenic responses.

9-04 A novel screen for mutants with reduced sensitivity to red light identifies new locus involved in phytochrome signaling

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Phytochrome plays an important role in the de-etiolation response of seedlings to red and far-red light. Mutant phenotypes indicate that Phytochrome A directs seedling de-etiolation in response to far red light while Phytochrome B is the major photoreceptor for red light. However, the *phya phyb* double mutant shows a stronger phenotype in red light than the *phyb* mutant, which suggests that phytochrome A also has a role in the red light response. Genetic screens for *Arabidopsis* seedlings with impaired de-etiolation have already identified a number of components of phytochrome signaling. In this study we have used a sensitized screen in a *phya* mutant background to identify potentially novel mutants hyposensitive to red light. We have isolated a new mutant with a long hypocotyl phenotype in red light, which is enhanced in the *phya* background. We have mapped the mutant gene and identified the lesion. The characterization of this mutant will be presented.

9-05 *FLC*: Giving plants a clock for all seasons

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The circadian clock times 24-hour biological rhythms, and responds to environmental light and temperature signals. An abundance of natural allelic variation exists in circadian clock genes of various *Arabidopsis* ecotypes. We previously identified *FLC* (*Flowering Locus C*) as a candidate for a circadian period QTL (Quantitative Trait Locus) in *Arabidopsis*. Here we show a dose dependent response of *FLC* expression on the circadian clock. Mutations of genes that regulate *FLC* levels also change circadian period. Interestingly, we further demonstrate that vernalisation treatment alters the circadian clock, in line with the down-regulation of *FLC*. We suggest that *FLCs* regulation of the clock is similar to its regulatory mechanism in the flowering pathway. *FLC* may be playing an adaptive role in the circadian clock in order to optimise flowering time. However, due to the physiological characteristics of *Arabidopsis*, little has been done to ascertain adaptive advantages of a circadian pacemaker in natural environments. To address this issue, we identified several circadian period QTL in the crop species *Brassica oleracea*, where a wide variety of field trial QTL data was available. We observed co-localisation of circadian QTL with previously identified QTL associated with water use efficiency (the measure of an organisms ability to fix atmospheric carbon for photosynthesis, as a ratio of water transpired). Our work represents the first evidence of a circadian clock in this vegetative crop species, and also gives an interesting handle on the significance of a circadian clock in the field.

9-06 A His-to-Asp phosphorelay modifies the activity of ARR4 on phytochrome signaling

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The *Arabidopsis thaliana* response regulator 4 (ARR4), expressed in response to phytochrome B (phyB) action, specifically interacts with the extreme N-terminus of the photoreceptor. ARR4 stabilizes the active Pfr form of phyB in yeast and in planta, thus elevates the level of the active photoreceptor in vivo. Accordingly, transgenic Arabidopsis plants overexpressing ARR4 display hypersensitivity to red light but not to light of other wavelengths. We therefore propose that ARR4 acts as an output element of a two-component system that modulates red light signaling on the level of the phyB photoreceptor. The two-component system is a signal transduction mechanism that requires a His-to-Asp phosphorelay to modulate the activity of a response regulator (e.g. ARR4). Consequently, we generated transgenic Arabidopsis lines expressing a non-phosphorylable form of ARR4 (ARR4D95N). These lines show hyposensitivity to light observed also for an ARR4 loss-of-function mutant. These results suggest that the action of ARR4 on phyB requires a His-to-Asp phosphorelay. By yeast two-hybrid approach and co-immunoprecipitation assays we identified the histidine phosphotransfer protein 1 (AHP1) to specifically interact with ARR4. Thus, AHP1 may mediate a phosphotransfer from different sensor histidine kinases to ARR4. Because the activities of multiple sensor histidine kinases are integrated at the level of AHPs, ARR4 may be a target of a complex two-component signaling network, which in turn regulates phy-dependent signal transduction. To understand, how this network functions, it is essential to identify the stimuli, and therefore, the sensor histidine kinases that initiate the AHP1-to-ARR4 phosphotransfer process.

9-07 Critical role for CCA1 and LHY activities in keeping circadian rhythmicity in Arabidopsis

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Plants have developed a circadian system that allows them to adapt to the dynamic changes in their environment, mainly in light quality and quantity. The environmental information must ultimately modulate the activity of the components of the molecular mechanism that generates the circadian rhythms, in order to keep it in synchrony with the seasonal day-night cycle. *CCA1* and *LHY* code for two highly similar transcription factors, each containing a single-MYB domain, involved in a negative feedback loop important for the functioning of the *Arabidopsis* circadian oscillator. The similar circadian phenotypes of lines overexpressing either *CCA1* or *LHY* have suggested that the functions of these two transcription factors are largely overlapping. *cca1-1* plants, which lack CCA1 protein, show a short period phenotype for the expression of several genes when assayed under constant light conditions. This suggests that LHY function is able to only partially compensate for the lack of CCA1 protein. It was, therefore, of great interest to obtain plants with a simultaneous loss of both functions, in order to determine the extent of the redundancy between CCA1 and LHY and to assess their collective role in the molecular clock mechanism. We have applied RNAi technology to obtain plants lacking both activities, *cca1-1 lhy-R*, and show that these plants are unable to maintain sustained oscillations in both, constant light and constant darkness. However, these plants exhibit some circadian function in light/dark cycles, showing that the *Arabidopsis* circadian clock is not entirely dependent on CCA1 and LHY activities.

9-08 Molecular analysis of an NBD-like subfamily of ABC proteins in Arabidopsis

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ABC proteins (for ATP-Binding Cassette) are a large family of active membrane transporters. They allow translocation of numerous substrates after Mg2+-ATP fixation and hydrolysis at the cytosolic nucleotide binding domain (NBD). In Arabidopsis thaliana, 129 ORF have been identified encoding ABC proteins. Among these, 11 correspond to NBD-like proteins, with no transmembrane domain. Very few studies have been done in eucaryotes about this ABC subfamily and all of them concern transcription or translation factors. The isolation of an insertional mutant has allowed us to study the expression pattern of the AtPOP1 gene, fused to the GUS reporter gene of the T-DNA. AtPOP1 is expressed in roots when plants are grown in light, and in hypocotyl when plant are grown in the dark. However, the northern analysis reveal and homogeneous expression in all tissues tested. Northern analyses also reveal transcription induction by sugars and a higher level of expression under light. AtPOP1 encodes for an NBD-like protein of 292 aa with high homology to the yeast CAF16 protein and to 2 additional proteins in Arabidopsis: AtPOP2 and AtPOP3. The three loci present the same transcriptional pattern in northern. Transgenic plants have been generated where the reporter GFP gene is under the control of AtPOP gene promoters. Such transgenics demonstrate that the tissue post-trancriptional regulation by light is conserved between the 3 loci. Complementation assay of yeast Caf16 deletion mutant is under way. Temporary transformation assays will be performed in order to determine the subcellular localization of the POP proteins.

9-09 Ectopic expression of tomato phytochrome genes in *Arabidopsis thaliana*

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The phytochrome (phy) chromoprotein photoreceptor family plays an important role in the regulation of plant growth and development in response to light. Like other dicotyledonous plants investigated tomato has five *PHY* gene family members. In *Arabidopsis* the genes are called *PHYA-E*. Analysis of transgenic plants overexpressing heterologous *PHY* genes has revealed a modification of plant architecture: induced dwarfism and reduced apical dominance and concomitant increase in branching. Both tomato and *Arabidopsis* have two *PHYB* family members: called *PHYB1* and *PHYB2*; *PHYB* and confusingly *PHYD*, respectively. These genes are not orthologues of each other and have arisen in the two families as a result of independent duplication of a progenitor *PHYB*. We have expressed tomato *PHYB1* and *PHYB2* in the *Arabidopis* wild type (L *er*) and *phyB* - null mutant (*hy3*, ABRC # CS6213) under the control of a 2X *35S* promoter. Both *PHYB1* and *PHYB2* transformants in the wild-type background result in a dwarf phenotype and are capable of rescuing the phenotype of the *phyB* mutant. Experiments under broad-band and monochromatic red light of different fluence rates suggest that the *PHYB2* overexpression is more efficient than *PHYB1*. We are currently determining the transgene expression and photoreceptor levels in each line. A comparison will be made of these results with data from plants having localized overexpression of the same genes in the epidermis using the *LTP* epidermal-specific promoter.

9-10 SPA1, a component of phytochrome A signal transduction, regulates the light signaling current

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Mutations in a component of phytochrome A (phyA) light signal transduction, *SPA1*, result in enhanced responsiveness of Arabidopsis seedlings to red and far-red light. Here, we have examined the effects of *spa1* mutations on the two known modes of phyA function, the high-irradiance responses (HIR) to continuous irradiation with far-red light and the very-low-fluence-responses (VLFR) to inductive pulses of light that establish only a small proportion of active phyA. *spa1* mutants exhibited an enhanced VLFR under hourly pulses of far-red light for hypocotyl growth inhibition, cotyledon unfolding, anthocyanin accumulation, block of greening in subsequent white light and negative regulation of phyB signaling. We provide evidence that the phenotype of *spa1* mutants in red light is also caused by an increase in the VLFR. Taken together, our results indicate that light-induced hypocotyl growth inhibition in *spa1* mutants is primarily due to a VLFR. While wild-type seedlings required hourly pulses of far-red light to induce a VLFR, infrequent irradiation with far-red pulses (every 12 h) was sufficient to induce a strong VLFR of hypocotyl elongation in *spa1* mutants. This shows that the effect of the VLFR was more persistent in *spa1* mutants than in wild type. We, therefore, propose that SPA1 has an important function in reducing the persistence of phyA signaling. *spa1* mutations also enhanced the HIR of anthocyanin accumulation and of phyA-mediated responsivity amplification towards phyB. Thus, our results suggest that *spa1* mutations amplify both phyA-mediated VLFR and HIR.

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9-11 HYH, a target of COP1 regulation

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Arabidopsis COP1 acts to repress photomorphogenesis in the absence of light. It was shown that in the dark, COP1 directly interacts with the bZIP transcription factor HY5, a positive regulator of photomorphogenesis, and promotes its proteasome-mediated degradation. Here we identify a novel bZIP protein HYH, as a new target of COP1. We identify a physical and genetic interaction between HYH and COP1 and show that this interaction results in dark specific degradation of HYH. Genetic analysis indicates that HYH is predominantly involved in blue light regulation of development and gene expression, and that the function of HYH in part overlaps with that of HY5. The accumulation of HYH protein, not the mRNA, is dependent on the presence of HY5. Our data suggests that HYH and HY5 can respectively act as heterodimers and homodimers, thus mediating light-regulated expression of overlapping as well as distinct target genes. We propose that COP1 mediates light control of gene expression through targeted degradation of multiple photomorphogenesis-promoting transcription factors in the nucleus.

9-12 The role of GRAS proteins in light signaling

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GRAS proteins are a recently discovered family of plant-specific proteins that play important regulatory roles in diverse aspects of plant development. The name "GRAS" derives from the first three members to be identified, GAI, RGA andSCR. We have identified an *Arabidopsis* mutant, *pat* (phytochrome A signal transduction) *1-1*, which shows strongly reduced responses in continuous far-red light. Physiological and molecular data indicate that this mutant is disrupted at an early step of phytochrome A signal transduction. The *PAT1* gene encodes a protein which shares all the signature motifs of GRAS proteins. Together with three other proteins (SCL5, 13 and 21) PAT1 constitutes a sub-group of the 32 GRAS proteins found in *Arabidopsis*. GFP fusions of all proteins of this sub-branch localize to the cytoplasm. Analysis of the function of the proteins in the "PAT1" sub-branch are carried out in antisense plants. The results indicate that these proteins are also involved in light signal transduction.

9-13 ATH1 integrates PhyB-signaling and GA-biosynthesis to regulate photomorphogenic responses

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Here we describe a functional analysis of *ATH1*, a light-regulated Arabidopsis homeobox transcription factor gene. *ATH1* is highly active in the shoot apical meristem (SAM) and leaf primordia during early stages of seedling development. Prior to floral transition, ATH1 SAM expression is gradually downregulated. Arabidopsis and tobacco plants with deregulated ATH1 expression display a flowering time phenotype and are specifically affected in phyB-mediated deetiolation and shade avoidance responses. In addition, ectopic expression of ATH1 causes semi-dwarfism that can be corrected by foliar applications of GA3. Quantitative GA analysis revealed that in severe overexpressors GA1 levels were dramatically reduced, due to a metabolic block of the conversion of GA19 to GA20 and probably also the one of GA20 to GA1. We conclude that ATH1 represents a positive mediator of a phyB-signalling and controls various aspects of photomorphogenesis by regulating the final steps of GA biosynthesis.

9-14 The *eid* mutants: A novel class of hypersensitive mutants involved in phytochrome A-dependent light signaling

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To perceive red and far-red light, plants have evolved specific photoreceptors called phytochromes. Even though the spectral properties of all phytochromes are very similar, they show a distinct mode of action. Phytochrome A (phyA) is adapted to sense very low light fluences and continuous far-red light. We used specific light programs to screen for mutants that lead to a hypersensitive phyA light response. Because all of our mutants exhibited an increased response towards continuous far-red light, they were called *eid* -mutants for empfindlicher im dunkelroten Licht which means more sensitive in far-red light. *eid6* was identified as a novel *cop1* allele (= constitutive photomorphogenesis) that does not exhibit a constitutive light response. *eid4* is a novel hypersensitive allele of phyA with altered light-driven protein degradation kinetics. EID1 is a negatively acting component of the signaling cascade that shifts the responsiveness of the phyA signaling system from red to far-red light. The expression of the Eid1 phenotype requires the presence of phyA. EID1 is a novel nuclear F-box protein that interacts with the Arabidopsis Skp1-homologs ASK1 and ASK2. Thus, EID1 is most probably a component of an SCF ubiquitin ligase complex. Because phyA degradation remains unaltered in *eid1* mutants, EID1 most probably acts by targeting activated components of the phyA signaling pathway to ubiquitin-dependent proteolysis. By genetic and physiological analyzes, we could demonstrate that EID1 and SPA1, another negative regulator of phyA responses, have distinct but overlapping functions in phyA-specific light signaling.

9-15 Characterisation of a novel mutant *hsp (hypersensitive signalling photoreceptor response)*, a common component in the phytochrome and cryptochrome signalling pathways in *Arabidopsis thaliana*

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Photomorphogenesis and the subsequent developmental programme are activated by distinct, information-transducing photoreceptors, which are sensitive to different regions of the electromagnetic spectrum. During de-etiolation and seedling establishment the red/far-red light-absorbing phytochromes and the blue light-absorbing cryptochromes jointly control the expression of a vast array of genes. This means that in the seedling, phytochrome and cryptochrome signal transduction must converge at common components. To identify such components we have screened for mutants that exhibit altered de-etiolation under different monochromatic light sources. One mutant isolate, *hsp*, has an enhanced de-etiolation phenotype that is specific to red and blue light. Thus, *hsp*, represents a completely novel class of mutant which may define an early convergence point for phytochrome and cryptochrome signalling. The recessive *hsp* mutation was caused by T-DNA insertion at a single locus. Progress on the characterisation of this mutant will be presented.

9-16 Phase-specific circadian clock regulatory elements in Arabidopsis

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The circadian clock enables an organism to phase aspects of its biology to precise times of day. The mRNA abundance for different clock-regulated genes peaks at distinct times; *CATALASE 2* (*CAT2*) and *CATALASE* 3 (*CAT3*) mRNA abundance peaks in morning and evening, respectively. In order to establish which *cis* -acting elements are responsible for phase-specific transcription, the *CAT3* promoter was resected and fused to luciferase for *in vivo* analysis. Progressive deletion of the *CAT3* promoter shows that an element necessary for evening-specific circadian transcription lies in the 25 bp region between -199 and -174 of the *CAT3* promoter. Within this region lies the Evening Element (EE: AAAATATCT), which differs by only one base from the CCA1 Binding Site (CBS: AAAAATCT) that has been implicated in morning-specific circadian regulated transcription. Site-directed mutation of the EE within the -281/+1 *CAT3* promoter context eliminated rhythmic transcription, confirming the necessity of the EE for evening-specific transcription. We tested the hypothesis that the EE and the CBS specify circadian phase by site-directed mutagenesis to convert the *CAT3* EE into a CBS. Changing the *CAT3* EE to a CBS changes the phase of peak transcription from the evening to the morning in continuous dark and in light-dark cycles, consistent with the specification of phase by the single bp that distinguishes these elements. However, rhythmicity of the CBS-containing *CAT3* promoter is dramatically compromised in continuous light. Thus, additional information normally provided in the context of a morning-specific promoter is necessary for full circadian activity of the CBS.

9-17 Characterization of phytochrome negative regulatory factor in Arabidopsis

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We have isolated an *am118* mutant showing phyB mutant phenotype from activation tagging mutagenesis of winter annual strain of *Arabidopsis* (*FRI-Col*). T1 generation of *am118* (*FRI*-Col) showed interesting phenotype, such as elongated hypocotyl and petiole, serrated and pale green leaves, and early flowering time. T2 population of *am118* (*FRI*-Col) showed 2.6:1 segregation ration for the mutant phenotype, suggesting *am118* (*FRI*-Col) is dominant. However, the mutant phenotype was not cosegregated with basta resistance. For genetic and molecular analyses of the mutant regarding phytochrome signaling, we introduced the *am118* (*FRI*-Col) to wild type Columbia (Col). The *am118* (Col) mutant was named as *dhy1* (*dominant long hypocotyls* 1). Physiological characterization showed that *dhy1* is deficient in a subset of phytochorme B-regulated responses, including the inhibition of hypocotyl and petiole elongation under red light and shade avoidance response. On the contrary, phytochrome A-regulated responses, including the inhibition of hypocotyl elongation, far-red-preconditioned blocking of greening, and induction of germination under far-red light were normal. When checked if *dhy1* has a defect in *PHYB* gene by DNA gel blot analysis and Western blotting experiment, *dhy1* was most likely to have normal *PHYB* gene. These results suggest that *DHY1* is a negative regulatory factor in phytochrome B signaling pathway. Genetic and molecular analyses are in progress to gain insights into the function of *DHY1* gene.

9-18 PKS1 (Phytochrome Kinase Substrate 1) and PKS2 are two repressors of phyA signalling with different light regulation

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The phytochrome binding protein PKS1 is phosphorylated by purified oat phytochrome A in a light-regulated fashion (Fankhauser et al. (1999) Science 284 : 1539). It belongs to a small multigenic familly of 4 basic proteins, PKS1 to PKS4. The expression of PKS1 and PKS2 is temporally and spacially light-regulated in a different way. Analysis of PKS1 and PKS2 mRNA levels and PKS1 protein levels of etiolated seedlings transferred into light revealed that PKS1 expression is transiently induced, whereas the induction of PKS2 expression is biphasic. Observation of transgenic lines carrying PKS1/PKS2 promoter::GUS and of PKS1 promoter::PKS1-GFP indicated that, in response to light, PKS1 expression is restricted to the elongation zones of the hypocotyl and of the root, and at the base of the cotyledons. On the other hand, after transition to light, PKS2 is expressed in the upper and in the lower part of the hypocotyl and in the cotyledons. Biochemical studies and microscopic observation of PKS1 promoter::PKS1::GFP lines showed that PKS1 is tighly associated to the plasma membrane. Physiological analysis of seedlings lacking or over-expressing PKS1 and PKS2 suggest that PKS1 and PKS2 act as repressors of the very low fluence response and that a balance between PKS1 and PKS2 is critical for this response.

9-19 Regulation of the nucleo-cytoplasmic partitioning of the phytochrome A, B, C, D and E photoreceptors and the role of induced nuclear speckle formation

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The phytochrome family of plant photoreceptors has a central role in the adaptation of plant development to changes in the ambient light environment. The individual phytochrome species regulate different or partly overlapping physiological responses. In transgenic Arabidopsis plants we have analyzed the intracellular localization of phytochrome-A to E:GFP fusion proteins. All phytochrome-GFP fusion proteins are transported in the nuclei in a light-dependent manner accompanied by the formation of subnuclear structures. Appearance of these nuclear speckles exhibits distinctly different kinetics and wavelength dependence and is also regulated by diurnal rhythm. Furthermore, we demonstrate that nuclear import of GFP fusion proteins of genetically characterised mutant phytochrome A and B photoreceptors is still regulated by light but is not accompanied by formation of nuclear speckles. These results suggests that (i) differential regulation of the translocation of phytochrome-A to E into the nuclei plays a role in the specification of functions and that (ii) appearance of speckles is a functional feature of phytochrome regulated signalling.

9-20 Light-dependent dynamics of the intracellular localisation of PIF3

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The phytochromes are plant photoreceptors that regulate gene expression and development throughout the whole life cycle of plants. After light treatment, these photoreceptors are transported into the nucleus and forming speckles. One intriguing question is, how light signaltransduction continous in the nucleus.It is reported, that PhyA and PhyB are interacting in vitro with PIF3 (Phytochrome interacting factor 3), a transciption factor. Point-mutations in the phytochrome-molecules prevent this interaction (Ni et al., 1998) as well as the formation of phytochrome-speckles (Kircher et al., 2002). So the speckle formation of the phytochromes can be correlated to their physiological function. We are testing under which conditions PIF3 could possibly interact with PhyA or PhyB in planta. To this purpose, we used transgenic plants containing PIF3::GFP-fusion-proteins to analyze the intracellular PIF3-localisation by fluorescent microscopy. Additionally, we looked for situations, where PIF3 is colocalizing with PhyA or PhyB. This approach will provide informations about the conditions, timewindows or stages in plant development, when PIF3 plays a role in phytochrome signaling.

9-21 Natural variation identifies genes that maintain the pace of the circadian clock

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Leaf movement analysis of 29 *Arabidopsis thaliana* accessions has revealed considerable natural variation of circadian period in response to temperature. Temperature compensation, the ability of the circadian clock to maintain its period close to 24 hours over a wide range of constant temperatures, is a defining feature of the endogenous oscillator. It is under natural selection in other species, but virtually nothing is known about this process in *Arabidopsis*. We have identified Quantitative Trait Loci (QTL) in temperature compensation based on genotype by environment interactions between the Landsberg *erecta* (L *er*), Columbia (Col) and Cape Verde Isles (Cvi) accessions. Col and Cvi by L *er* recombinant inbred populations were assayed for circadian leaf movement period at three temperatures, identifying 6 QTL with temperature specific period effects. These QTL were confirmed by analysis of near isogenic lines, and likely resolved an epistatic interaction between two chromosome 1 QTL. A strong QTL, with a 1.3 hour 12 degrees C specific period effect, mapped away from previously known clock genes onto chromosome 5. This QTL in part accounts for improved temperature compensation in the Cvi accession at low temperature. Another QTL, which maps to a previously identified gene, counteracts period shortening at higher temperatures. Mutant alleles of this gene display responses consistent with it being an important component of circadian temperature compensation at higher temperatures. In contrast to the *Drosophila* and *Neurospora* clock models, our QTL implicate genes outside the proposed central oscillator of *Arabidopsis* imparting temperature compensation on the clock**.**

9-22 A Role for *ABI3* (*Abscisic Acid-Insensitive 3*) In Arabidopsis Circadian Regulation

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The *ABI3* gene was originally identified in a screen for mutations that allowed germination in the presence of abscisic acid (Koorneef *et al.*, 1984). Mutation of the *ABI3* gene results in embryos that do not fully complete maturation and express characteristics of seedlings. The *ABI3* gene encodes a transcriptional regulator that was initially thought to function in an embryo-specific manner. However, recent studies have shown a role in vegetative tissues (reviewed in Rhode *et al.*, 2000). Furthermore, *abi3-4* null mutant plants flowered earlier than wild-type plants, both under long and short day conditions (Kurup *et al.*, 2000). A screen for protein partners of ABI3 identified several ABI3-interacting proteins (AIPs). One of the partners identified was TOC1, a putative component of the circadian oscillator (Kurup *et al.*, 2000). This observation led to the hypothesis that ABI3 may play a role in the circadian regulation of gene expression. To test this hypothesis, rhythmic leaf movements were analysed in plants with disrupted ABI3 function. Interestingly, *abi3* mutant seedlings showed disrupted rhythms. The strong loss of function alleles *abi3-4* and *abi3-6* altered the period of rhythmic leaf movement in opposite directions compared to the relevant wild-type controls (L*er* and Col respectively). However, the overexpression of *ABI3* did not significantly alter leaf movement rhythms. These results suggest a possible role for *ABI3*, perhaps via an interaction with *TOC1*, in the mechanism of the Arabidopsis circadian clock.

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9-23 Investigating a model for the circadian oscillator in Arabidopsis

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The rhythmically expressed genes, *LATE ELONGATED HYPOCOTYL* (*LHY*), *CIRCADIAN CLOCK ASSOCIATED 1*(*CCA1*) and *TIMING OF CAB1* (*TOC1*) are proposed to form an oscillatory feedback loop that is essential for clock function in Arabidopsis. Alabadi *et al.* (Science, 2001, 293:880-3) have proposed a model for the central oscillator in which *LHY and CCA1* negatively regulate *TOC1,* whilst *TOC1* activates *LHY* and *CCA1.* The *LHY* and *CCA1* genes encode MYB-like transcription factors that regulate their own transcription and that of each other. To test whether *LHY* and *CCA1* have an essential function in the circadian clock, we assayed the effects of loss of function mutations on the expression of rhythmically expressed luciferase reporter genes. Both single mutants (*lhy-11 and cca1-1*) exhibited circadian rhythmicity, but their period was shorter than wild-type plants. These similar phenotypes suggested that *LHY* and *CCA1* might have redundant function in the clock. In the double mutant (*lhy-11cca1-1*), gene expression rhythms damped rapidly, both in constant light and in constant darkness. This light-independent phenotype indicates that *LHY* and *CCA1* do not simply mediate light input to the clock. This supports their proposed function in the oscillator. Surprisingly, levels of *LHY::luc* and *CCA1*::luc expression were not increased in *lhy-11* and *cca1-1* plants, and expression of *CCA1::luc* was dramatically reduced in *cca1-1*. This result indicates that *CCA1* function is required to promote its own expression. Thus, the circadian clock of Arabidopsis comprises a positive feedback loop, in addition to the negative feedback loop mediated by *LHY*/*CCA1* and *TOC1*.

9-24 Quantitative genetic analysis for seedling photomorphogenesis in Arabidopsis

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Seedling development plays a central factor for successfully establishment of the plant. Light is a crucial environmental signal that modulates the de-etiolation processes such as chloroplast development, inhibition of hypocotyl length and cotyledon unfolding. In this study we use the wide genetic variation in Arabidopsis to dissect the genetic control of the inhibition of hypocotyl and cotyledon unfolding. To reach this objective we analysed both responses in Ler x Cvi recombinant inbred mapping populations (RILs) to daily far-red pulses (FRp), red pulses (Rp) and far-red continuous (FRc). We found two important quantitative trait loci (QTLs), one associated to the cotyledon unfolding in the top of the chromosome 1 (*COT1*) and another associated to the cotyledon unfolding and inhibition of the hypocotyl in the middle of the chromosome 2. Near-isogenic lines (NILs) and cry2-transgenic seedlings allow us to establish that the previously described natural variation at the cryptochrome 2 gene (El-Assal et al., 2001) is responsible for the cotyledon unfolding in *COT1*. The physiological analysis of two NILs differing only in some alleles in the chromosome 2 suggests that both photomorphogenic responses are controlled by two near located loci, however none of those are associated with the *ERECTA* locus. Finally, we found three minor QTLs associated with the inhibition of hypocotyl length under FRp indicating that these loci could be responsible for the VLFR (very-low-fluence response).
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9-25 Isolation and characterization of a phytochrome C mutant in Arabidopsis

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In Arabidopsis, phytochromes (phy) are encoded by 5 genes, *PHYA* through *PHYE*. Mutants defective in phytochrome species now exist for phyA, phyB, phyD and phyE. Mutant analysis shows that phyA and phyB have major roles in controlling seedling deetiolation under continuous red (Rc) and far red (FRc) light: *phyA* is blind to FRc, indicating that phyA is exclusively responsible for inhibiting hypocotyl elongation under FRc light, and *phyB* is greatly impared in sensing Rc light, indicating that phyB is the predominant phytochrome species mediating the inhibition of hypocotyl elongation under Rc light. PhyD also participates in this process but in a redundant manner to phyB, since *phyD* has normal sensitivity to Rc but the double mutant *phyBphyD* is more hyposensitive to Rc than *phyB*. Recent isolation and characterization of *phyE* has established that phyE is not involved in the control of seedling deetiolation in Arabidopsis.Isolation of an Arabidopsis *phyC* mutant in forward genetics screens has so far been elusive. To study the role of phyC in the control of seedling deetiolation, we have taken a reverse genetics approach to isolate *phyC* mutants in Arabidopsis. We have screened different mutant collections and have isolated 2 different T-DNA insertional mutant alleles and 1 fast neutron deletion allele. We are currently characterizing the deetiolation response of the mutant alleles under Rc and FRc light.

9-26 Using natural variation to isolate an enhancer of *early flowering 4*.

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Photoperiod or daylength is an environmental signal commonly used to trigger the switch from vegetative to reproductive growth in plants. Studies of daylength perception suggest a strong link between this process and the plant circadian clock. We have recently isolated *EARLY FLOWERING 4 (ELF4)* a gene that affects the circadian clock and the ability of plants to sense daylength. The early-flowering phenotype of *elf4* mutants is most easily distinguished under non-inductive short day conditions (8 hours light: 16 hours dark). The *elf4* mutant was isolated from a T-DNA mutant collection in the Ws ecotype. When introgressed into Landsberg *erecta* (L *er*), *elf4* mutation leads to significantly earlier than *elf4* in Ws. Approximately 1/16 of the original F2 population (*elf4* (Ws) X L *er*) displayed enhanced early-flowering suggesting that this phenotype is the result of a single recessive locus in L *er*. Using a positional cloning approach, the enhancer locus has been mapped to chromosome V in a region that does not include genes known to affect flowering time or circadian rhythms. Identification of this locus will hopefully lead to a better understanding of photoperiodism as well as provide further insight into the events that may have led to the optimization of flowering time in Arabidopsis in different geographic regions.

9-27 HFR1, a phytochrome A-signaling component acts in a separate pathway from HY5, downstream of COP1 in *Arabidopsis thaliana*

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HFR1, a basic helix-loop-helix protein, has been shown to be required for a subset of phytochrome A (phyA)-dependent photoresponses. To further investigate the role of HFR1 in light signaling, we have examined the genetic interaction between HFR1 and HY5, a positive regulator of light signaling, and COP1, a repressor of photomorphogensis. Double mutant analysis suggested that HFR1 mediates phyA-dependent inhibition of hypocotyl elongation independently of HY5. HFR1 was shown to be necessary for a subset of *cop1*-triggerred photomorphogenenic phenotypes in the dark, including inhibition of hypocotyl elongation, gravitropic hypocotyl growth, and expression of light-inducible genes, *CAB* and *RBCS*. Phenotypic analysis of the triple mutant *cop1hy5hfr1* indicated that both HFR1 and HY5 are required for *cop1*-mediated photomorphogenic seedling development in darkness, consistent with their additive roles in phyA-dependent signalling. Taken together, these results suggest that HFR1 might act downstream of COP1, in a separate pathway from HY5, to mediate photomorphogenesis in Arabidopsis.

9-28 Analysis of blue-light signaling pathways using a *cry1cry2phot1phot2* quadruple mutant.

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Light is the most effective environmental stimuli to plants. Blue light receptors were identified and investigated next to red/far-red light receptor, phytochrome. Blue light receptors consist of two types of proteins, cryptochrome and photoropin. Previous reports suggested that cryptochromes, CRY1 and CRY2 function mainly in photomorphogenesis, such as inhibition of hypocotyl elongation and floral induction and that on the other hand, phototropin, PHOT1 and PHOT2 mainly regulate blue light-mediated-movement such as phototropism, chloroplast movement and stomatal opening. Each receptor in the same type has functional redundancy as well as functional peculiarity in meeting fluence rate. Recently it has been suggested that cryptochrome and phototropin might also have a little redundancy in some responses. To remove the possibility that these redundancies might hide the original function of each receptor, we applied "gain-of-function" analysis; blue light responses of *cry1cry2phot1phot2* quadruple mutant were compared with those of triple mutants or complemented quadruple mutants. We combined this analysis with physiological analysis or microarray analysis and then tried to reveal hidden function of each receptor as well as the relationship of four blue light receptors in signal transduction. The physiological analysis data suggested that no other blue light receptors may involve in some responses and that cryptochrome and phototropin have functional redundancy to some extent. The results of gene expression analysis using microarray suggest that two cryptochromes may regulate gene expression in overlapping manner through the same cis regulatory system. On the other hand, two phototropins showed little involvement in gene expression. This is the new strategy to shed light on the complicated mechanism regulated by photoreceptors.

9-29 The circadian regulated *GIGANTEA* gene and photoperiodic flowering

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The behaviour and reproduction of organisms is synchronised to favourable seasons of the year. Day length (photoperiod) is used by many of them as a seasonal signal. The *GIGANTEA* (*GI*) flowering-time gene promotes flowering in response to long day lengths. It is also needed for robust circadian rhythmicity and light signalling. *GI* encodes a large (1173 aa), plant-specific, nuclear protein of unknown function. *GI* transcript levels have a daily rhythmic pattern of expression (peaking 8-10 h into light), strictly regulated both by the circadian clock and by day length. We are studying the importance of *GI* timing by tracking GI protein accumulation through different day/night cycles using GI antibodies and immunoblotting. We will also test whether the GI protein is differentially activated indifferent day lengths by phosphorylation. In order to gain insight into GI function, we have identified a potential protein interactor, a BEL-1 LIKE protein, with the C-terminal segment of the GI protein using the yeast two-hybrid assay. In vitro binding experiments show binding between this protein and GI. Experiments are being carried out using T-DNA knock out lines to test the biological relevance of this protein interaction for the day length control of flowering. Finally, genetic screens for suppressors of the gi mutation are underway to identify downstream targets of GI or genes whose products interact with GI. Current progress of these experiments will be presented.

9-30 Blue and red light differently influence organization of actin cytoskeleton in *Arabidopsis* leaf mesophyll cells

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Structural association between chloroplasts and actin cytoskeleton was visualized by fluorescent staining of actin filaments with Alexa Fluor 488 phalloidin in pre-fixed, fully developed leaves of *Arabidopsis thaliana*. Prior to fixing, the samples were either dark-adapted or irradiated with blue or red light of equal photon fluence rates. Blue light irradiances were chosen that saturate the weak-light movement response of chloroplasts and nearly saturate their strong-light i.e. avoidance response. No dramatic changes of actin architecture were observed in strong as compared with weak light-irradiated or dark-adapted probes. A slight tendency to form fine network of filaments was found in the leaves irradiated with weak blue light. Similar patterns of actin cytoskeleton were obtained in leaves of wild-type *Arabidopsis* and in those of *phot2* photoreceptor mutant that lacks the avoidance response of chloroplasts. Opposite tendency was observed in the red-irradiated mesophyll: there, a finer network appeared more often in the cells treated with strong light than in those irradiated with weak light. Changes of actin organization caused by red light were particularly conspicuous in the leaves of the *phot2* mutant. The red light effect depended on the age of the plant and was absent in very young plants.These results suggest that different types of actin reorganization accompany chloroplast responses and other intracellular movement (cytoplasmic streaming ?) in the mesophyll cells of higher land plants. It may also be speculated that the absence of PHOT2 promotes phytochrome activity in these cells.

9-31 Genetic and cell-biological approaches to understand light control of chloroplast biogenesis

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Chloroplasts develop in angiosperms exclusively in the light. At least one quarter of all phytochrome regulated genes during early seedling development are photosynthesis-related. A plastid-to-nuclear communication signal is also required for the expression of photosynthetic nuclear genes. We are trying to understand whether a phytochrome signalling pathway specific for chloroplast biogenesis exists. Using a combination of plastid-defective *cue* Arabidopsis mutants, tobacco lines expressing a gymnosperm (non light-requiring) *Lhcb* photosynthetic promoter, and gymnosperm (pine) seedlings, we have shown that plastid signals are required also in the dark, for the basal level of expression of angiosperm *Lhcb* and the high level of expression of gymnosperm *Lhcb* in both tobacco and pine. These data are consistent with a model in which plastid signals play the highest hierarchical regulatory role, and phytochrome requirement for photosynthetic gene expression arose by repression in angiosperm promoters of the ability to respond to plastid signals in the dark. Separately, in collaboration with K. Pyke (Nottingham) and J. Gray (Cambridge), we have obtained data suggesting that an essential plastid signal is an activator of nuclear photosynthetic genes when plastids are viable, because plastid-less guard cells arising occasionally in the plastid division mutant *arc6* fail to express a photosynthetic nuclear gene marker. Finally, light quantity signals play a major role in the final composition of the mature Arabidopsis chloroplast, and we are currently analysing the extent of control of photosynthetic nuclear genes by chloroplast-generated light quantity signals, and whether there is an additional involvement of phytochromes or other photoreceptors.

9-32 PAP1/IAA26, a phytochrome interacting protein belonging to AUX/IAA family members, regulates PHYA-induced *CHS* expression through HY5.

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Red and far-red lights, perceived by phytochromes, are important external signals for the plant growth and development. Though many phytochrome signaling components including phytochrome-interacting proteins have been identified, the signal transduction pathways are still elusive. We have isolated IAA26 as a new phytochrome interacting protein from the yeast two hybrid screening (named as PAP1/IAA26). We show that phytochromes interact with PAP1/IAA26 and the Pfr form of phytochrome preferentially phosphorylates PAP1/IAA26 in vitro. The yeast two hybrid data showing the interaction between PAP1/IAA26 with other AUX/IAA family members suggest a possibility that phytochromes are in close contact with the AUX/IAA dimer network in the plant. To determine the physiological role of PAP1/IAA26 in the phytochrome signal transduction, we analyzed a T-DNA inserted mutant and a transgenic plant overexpressing PAP1/IAA26. The analyses indicate that PAP1/IAA26 is a positive signaling component for the PHYA-induced *CAB*, *CHS*, and *HY5* expression, but does not regulate the inhibition of hypocotyl elongation by light. We further show that the overexpression of CHS in the PAP1/IAA26 transgenic plant requires the functional HY5. Our data indicate that PAP1/IAA26 regulates PHYA-induced *CHS* expression through HY5.

10-01 Molecular and evolutionary analysis of GS-OH: Variable mRNA expression controls production of 2-hydroxy-but-3-enyl, the goiter causing glucosinolate

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Glucosinolate biosynthesis is commonly divided into a tripartite biosynthetic pathway. The first step involves carbon chain elongation of the base amino acid. The elongated amino acid then enters the second step where the common glucosinolate backbone structure is formed. The final step is structural modification of the glucosinolate side-chain. Structural variation can be incorporated in either the chain elongation or in the side-chain modification steps. Arabidopsis thaliana contains naturally occurring knockout mutations in side chain modification enzymes. This variation controls the plants glucosinolate profile and resistance to insect herbivory. One of these variable enzymes is GS-OH, which controls the production of 2-hydroxy-but-3-enyl glucosinolate or pro-goitrin. This glucosinolate has been implicated in the generation of mammalian goiter disease. Mapping natural variation for the presence or absence of 2-hydroxy-but-3-enyl glucosinolate identified a single 2-oxoacid dependent dioxygenase that catalyzes the production of 2-hydroxy-but-3-enyl glucosinolate from but-3-enyl glucosinolate. This gene contains unique promoter elements whose promoter position appears to control GS-OH expression. Further, phylogenetic evidence suggests that this enzyme arose early in A. thaliana speciation. Interestingly, phylogenetic and biochemical analysis indicates that GS-OH may have independently evolved three times in the Capparales. The evolutionary, molecular and biochemical data will be presented in addition to data on how GS-OH impacts insect herbivory.

10-02 Natural variation of non-photochemical quenching in Arabidopsis

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Natural variation of non-photochemical quenching (NPQ), a phenomenon of dissipating excess light energy into heat, was investigated. Based on NPQ of chlorophyll fluorescence, 50 Arabidopsis ecotypes were classified into high and low NPQ ecotypes. Among them, Ll-1, Sf-2 and Wen-0 were chosen as high NPQ ecotypes, and Col-0 and Ws were used as low NPQ ecotypes for further studies. The NPQ phenotypes were heritable, and no maternal effects seem to be involved in NPQ. Although Ll-1, Sf-2, and Wen-0 exhibited higher NPQ than Col-0 and Ws, all of them showed similar development until NPQ was measured. Additionally, pigment analysis by HPLC demonstrated that Ll-1, Sf-2, Wen-0, Col-0 and Ws have similar levels of xanthophyll pigments de-epoxidation states, one of known factors involved in NPQ, after being exposed to high light. The DNA sequence of the *PsbS* genes from Ll-1, Sf-2 and Wen-0 were nearly identical to each other, but they differed from those of Col-0 and Ws. However, the PsbS amino acid sequences of all five ecotypes were identical to each other, and these ecotypes contained very similar levels of the PsbS protein. To identify the factors causing the high NPQ phenotype, we made crosses between Ll-1 and Col-0 as well as Sf-2 and Col-0. All F1 plants from both reciprocal crosses showed slightly higher NPQ than Col-0, but significantly lower NPQ than the high NPQ parent. The F2 plants showed continuous segregation of the NPQ phenotype indicating that the NPQ phenotype is governed by multiple loci. These plants will be used to determine other factors involved in NPQ to understand detailed mechanisms of NPQ.

10-03 Conservation and divergence of regulatory elements of the floral homeotic gene *AGAMOUS*

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In flowering plants, the homeotic gene *AGAMOUS* (AG) and its orthologs control the development of the reproductive floral organs, stamens and carpels. In the reference plant *Arabidopsis thaliana*, sequences necessary and sufficient for *AG* expression in the center of flowers are located in the second intron, which is about 3 kb in size. This intron contains binding sites for two transcription factors, LEAFY (LFY) and WUSCHEL (WUS), which are direct activators of AG, as well as several other putative regulatory elements. We have used phylogenetic footprinting along with functional tests to examine the conservation and divergence of *cis*-regulatory elements in the *AG* intron. Although there is little obvious sequence conservation outside the Brassicaceae, except for an AAGAAT box and a pair of CCAATCA boxes, the intron from cucumber *AG* has at least partial activity in *Arabidopsis*. Within the Brassicaceae, several other motifs, but not the LFY and WUS binding sites previously identified, are highly conserved. The significance of these other conserved motifs has been confirmed by reporter gene analysis.
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10-04 Approaches for the identification of quantitative trait loci

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We have previously surveyed Arabidopsis accessions for variation in hypocotyl length light response (Maloof et al, 2001) and performed Quantitative Trait Locus (QTL) analysis in the Cvi/Ler RILs (Borevitz et al, 2002). Major QTL include a novel locus *LIGHT1*, and a locus where *PHYTOCHROME B* (*PHYB*) is a candidate gene. Sequence variation at *PHYB* revealed several candidate amino acid changes however less than would be expected had *PHYB* been evolving neutrally (McDonald-Kreitman: p=0.027). Two nonsynonymous changes in *PHYB* are significantly associated with hypocotyl length across accessions. A survey of 163 genome wide SNPs provided no evidence of long range linkage disequilibrium (LD) nor of population structure among 73 accessions, indicating that LD mapping could be used to fine map QTL (Nordborg et al, 2002). In addition SNP108 was significantly associated with hypocotyl length and mapped to *LIGHT1*. Results from a high resolution LD mapping study at *LIGHT1* will be presented. If QTL are determined by gene expression variation, this could be detected using global transcription analysis. We profiled three day old seedlings of the *LIGHT1* NIL and parental L*er* and Cvi lines. Significant transcription variation, mapping to the *LIGHT1* interval, was identified. In order to identify thousands of markers for genome wide LD and traditional mapping and to remove hybridization noise from transcription analysis across accessions we developed a genotyping method using Affymetrix GeneChips. Nearly 4000 Single Feature Polymorphisms (SFPs) were identified between Col and L*er* at a 5% false positive rate. Bulk segregant analysis with chip genotyping was used to map a known mutation. 105 potential gene deletions were identified, including transposons, disease resistance genes (R genes), genes involved in secondary metabolism, and other potential QTL candidate genes. Linkage Disequilibrium mapping, transcription profiling, and chip genotyping techniques are being used to identify QTL.

10-05 Transcription regulation of natural variation among Arabidopsis ecotypes, implications of evolutionary dynamics

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Transcription and translation of genes are key components in genome evolution. In order to understand molecular basis of natural variations among different Arabidopsis ecotypes, we used GeneChip microarray to analyze the changes of more than 8300 genes at genomic sequence level as well mRNA expression level. Through comparative genomic hybridization, variations within the gene coding sequences among the five ecotypes, Col-0, C-24, L *er*, WS-2, and NO-0 were identified. Examination of these variations suggested that the majority of them were single feature polymorphisms and small insertions or deletions. Using the results obtained from genomic DNA hybridization as an internal control, relative mRNA abundance of about 7000 genes from the five ecotypes in different organs, and/or at various ages was determined by RNA microarray analysis. Cluster analysis of RNA expression data revealed a general similarities in gene expression profiles among different ecotypes, as illustrated by the similarity between Col-0 and C-24, which correlates well with the phylogenic relations based on the coding sequence similarity. However, large expression differences of a number of genes in senescent leaves and siliques between C-24 and Col-0 were observed, suggesting that the transcriptional regulation towards late development stages of these two closely related accessions might be different. In addition, cluster analysis has also uncovered major differences between axis-origin organs such as roots, and auxiliary-origin organs such as leaves, two major organ origins during ancient plant landing. These differences are much more significant than the differences among different ecotypes. In order to understand the evolutionary dynamics of the Arabidopsis genome, correlation analyses of expression patterns of of about 6000 genes in examined accessions were conducted. A total of 93 most plastic genes with distinct expression pattern in each of the five ecotypes were identified. Among those are genes which serve as environmental sensors, such as *PhyB*. Furthermore, in order to exploit the role the environment plays during the occurrence of natural genetic variation, further analysis of expression patterns of the selected genes among different ecotypes, and the expression patterns of their paralogs were performed. The identification of genes which are highly variable among different accessions as compared to the differences among different paralogs suggests that environment has played an essential role in the regulation of gene expression. Our results provided rich information for modeling of genetic networks and their evolution.

10-06 A genomic survey of polymorphism in Arabidopsis

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Surveys of DNA sequence polymorphism in *Arabidopsis thaliana* have revealed that linkage disequilibrium is extensive but decays within 1 cM. When coupled with the high level of variability and the availability of inbred lines, this finding suggest that a haplotype map of *A. thaliana* could be extremely useful for mapping loci responsible for natural variation. We are constructing construct such a map by sequencing approximately 2000 short (500-700 bp) fragments distributed throughout the genome in a sample of 96 accessions. This means on the order of four fragments per cM, or one fragment every 50 kb. We are also developing a database and bioinformatics tools to make the data available and useful. The first half of the plate of 96 accessions will consist of stock center accessions, chosen so as to include most accessions that are currently being used in developing recombinant inbred lines (RILs) or in SNP-detection projects. The reminder of the sample will consist of a stratified sample of several freshly collected accessions from each of a number of populations in Europe and the USA. The purpose of this is to get further insight into the population structure of *A. thaliana*. Seeds from the chosen accessions will be made available through the stock centers.

10-07 Naturally-occuring variation for freezing tolerance in Arabidopsis

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Arabidopsis plants can survive freezing temperatures when previously exposed to low chilling temperatures. In order to identify the genetic determinants responsible for adaptation to freezing conditions we have searched for natural variation in freezing tolerance among several Arabidopsis ecotypes. Most of the analysed ecotypes developed high levels of freezing tolerance after exposure to low temperature during one week. However, the ecotype Cvi, from the Cape Verde islands, showed an impaired development of freezing tolerance under the same conditions as well as a reduction in the expression of cold regulated genes. A quantitative genetic analysis performed on recombinant inbred lines derived from the cross between Ler and Cvi indicated that differences in freezing tolerance between these two ecotypes can be mainly attributed to QTLs on chromosomes 1, 4 and 5. Analysis of the Arabidopis genome sequence around the QTL on chromosome 4 identified the *CBF* genes encoding the *C*-repeat/DRE *B*inding *F*actors as candidate genes responsible for this QTL. This hypothesis is supported by the nucleotide sequence polymorphisms between Ler and Cvi characterised in this region as well as the complementation of the freezing sensitive phenotype with a cosmid containing the *CBF* genes.

10-08 The characterization of seed dormancy genes in the Arabidopsis ecotype Cvi

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Research on seed dormancy in Arabidopsis has been hampered by the relatively low level of dormancy in freshly harvested seeds of the commonly used laboratory strains Landsberg *erecta (L er)* and Columbia. However, there are other accessions such as Cape Verde Islands (Cvi), which show a very strong dormancy. A set of recombinant inbred lines (RIL) derived from the cross L*er* x Cvi has been used to identify and locate quantitative trait loci (QTL). This analysis revealed 7 loci accounting for the seed dormancy genetic variation. Near isogenic lines (NILs) in a L*er* background have been developed to characterise the individual loci. For that, the germination behaviour of these NILs was analysed under different environments including hormone treatments. We have initiated the further fine mapping and genetic and physiological characterisation of the strongest QTL, located in chromosome 5 and referred to as *DOG1* (*d*elay *o*f *g*ermination). In addition, we have performed mutagenesis on the NIL carrying Cvi alleles at this QTL, and we have isolated a complete non-dormant mutant, C119. Genetic analysis of C119 showed that is completely linked to *DOG1* strongly suggesting that is a loss of function allele of *DOG1*.

10-09 Variation in growth rate between *Arabidopsis thaliana* ecotypes is correlated with cell division: Cell cycle and QTL analyses

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We used a kinematic analysis to investigate the growth processes responsible for variation in primary root growth between 18 ecotypes of Arabidopsis thaliana. Root elongation rate differed fourfold between the slowest (Ler; 71 um h-1) and fastest growing line (Ws; 338 um h^{-1}). This difference was contributed almost equally by variations in mature cortical cell length (84 um (Ler) to 237 um (Ws)) and rate of cell production (0.63 cell h^{-1} (Nw) to 1.83 cell h^{-1} (Ws)). Cell production in turn was determined by variation in cell cycle duration (19 hr (Tsu) to 48 hr (Nw)) and, to a lesser extent, by differences in the number of dividing cells (32 (Wei) to 61 (Ws)). We found no correlation between mature cell size and endoreduplication, refuting the hypothesis that the two are linked. There was however a strong correlation between cell production rates and the activity of the cyclin dependent kinase, CDKA. The level of the protein could explain 32% of the variation in CDKA activity. It is therefor likely that regulators of CDK activity, such as cyclins, inhibitors, and/or phosphorylation/dephosphorylation events, are also involved. These data provide a functional link between cell cycle regulation and whole plant growth rate as affected by genetic differences. In a quest to identify the nature of these genetic differences, we identified several QTLs associated with specific growth parameters. Based on these QTLs we hope to isolate the genes responsible for the observed growth differences and analyse their function.

10-10 QTL analysis in the Recombinant Inbred Lines derived from the L*er* x Cvi cross of *Arabidopsis thaliana*

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We have studied QTL controlling various morphological and developmental traits in the Recombinant Inbred Lines (RILs) derived from the *Landsberg erecta* x *Cape verdi islands* (Ler x Cvi) cross of *Arabidopsis thaliana*.We have also genotyped the RILs for an additional 16 microsatellite markers and mapped them with some selected AFLP (Amplified Fragment Length Polymorphism) markers that were already published. QTL were located by the Marker Regression approach using the QTL Cafe (http://web.bham.ac.uk/g.g.seaton/). A total of 21 QTL were found on just 3 chromosomes (none were locted on chromosome 3 or 4) for 13 out of the 16 traits analyzed, but many of these probably reflect the same QTL at different growth stages. Ler alleles determined earliness for both QTL governing flowering characters. Three QTL that control height were found on chromosomes 1, 2 and 5; the Cvi alleles increasing height of chromosomes 1 and 2 but decreasing on chromosome 5. Hence, the genes governing flowering characters showed a peculiar association while the genes seemed to be dispersed between Ler and Cvi for height characters. The QTL on chromosome 2 corresponds in position to the *erecta* mutation in Ler. The QTL for height on chromosome 5 has a larger effect on Cvi than *erecta* at early growth stages. Most QTL affect several traits.

10-11 Analysis of the genetic bases of nitrogen use efficiency in the model plant *Arabidopsis thaliana*

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Improving plant efficiency in nitrogen uptake and use requires a better knowledge of plant nitrogen assimilation pathway and its regulation. In our laboratory, a physiological and molecular studies of nitrate transport (see Abstract from Orsel, Krapp, Gojon, Daniel-Vedele) and a quantitative genetic studies of nitrogen use efficiency were performed on the plant model Arabidopsis. Arabidopsis offers unequivocal genetic advantages for QTL mapping and cloning purposes. A population of 420 recombinant inbred lines (F6) was derived from the cross between two genetically distant ecotypes, Bay-0 and Shahdara, that are adapted to contrasted habitats. A microsatellite-based genetic map was constructed from this population.
Several characters allowed us to study the variability for nitrogen use efficiency. These traits were measured in two contrasting nitrogen environments, one of which strongly limited plant growth. We mapped a total of 117 QTLs, which should represent at least 30 polymorphic genes partially controlling one or several characters.
The Bay-0 x Shahdara population proved to be very efficient for the genetic dissection of complex traits, the quality of the segregation and the precision of the genetic map. These QTLs highlight the strong interaction between genetic regulations and nitrogen nutrition level. Four loci seem particularly interesting in terms of nitrogen use efficiency variability: each of these loci represents a specific source of simultaneous variations for shoot dry matter, total nitrogen percentage, nitrate and and amino-acids contents.
The fine-mapping and positional cloning of these major loci should bring an interesting and comprehensive light on genes involved in the regulation of nitrogen use efficiency.

10-12 Natural genetic variation in Arabidopsis: A core collection of genotypes for high-throughput SNP discovery

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High throughput genotyping of SNPs using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has been proven for human samples to be a powerful approach for looking at natural genetic variation and for conducting association studies. At the Centre National de Génotypage, we are applying this method to the study of the natural variation present among *Arabidopsis* accessions (ecotypes) and particularly with respect to their response to different abiotic stresses. In order to increase the efficiency of SNP detection, we have defined a core collection of *Arabidopsis* accessions. Ten gene fragments of ca. 600 bp (introns and exons), distributed throughout the genome, were sequenced on 94 or ca. 240 *Arabidopsis* accessions from the different stock centers. SNP detection was performed using Genalys software, and the core collection chosen based on these results using the program MSTRAT. This core collection is to be used for SNP detection in candidate genes involved in responses to abiotic stress, following which the entire collection of *Arabidopsis* accessions will be genotyped for selected SNPs. Here we present the core collection, the expected efficiency of our sampling strategy and some of the salient features of the structuration of *Arabidopsis* genetic diversity that we have remarked in generating this collection.

10-13 The use of microsatellite DNA markers to investigate the marker variation and genetic diversity in natural populations of *Arabidopsis thaliana*

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Ten microsatellite markers were used to analyse marker variation (as defined by loci repeat number) and genetic diversity amongst 33 accessions of *Arabidopsis thaliana*. For convenience accessions were grouped together according to geographic origin: West Europe, East Europe, South Europe, North Europe, North America, Africa, and Asia. AMOVA analysis revealed no global significant difference in marker variation between and within geographic origin groupings. Marker variation between loci within accessions was shown to be highly significant. An average of 8.2 alleles per locus was detected contributing to a genetic diversity value of between 0.68 and 0.91. The distribution of repeat number was centred on the mean for most loci, however this distribution could not be regarded as normal. Linkage disequilibrium between loci was tested and shown to be highly significant. A phenetic tree based on absolute distance values (DAD) clustered the 33 accessions into 3 groups with no significant association between accessions and their geographic origin. The flowering time of all 33 ecotypes was also recorded. Regression analysis was performed to test the relationship between allele size at all 10 loci and flowering time. No significant relationship between allele size and flowering time was revealed. These results suggest that the populations studied have only recently been established. This is in agreement with current opinion that *Arabidopsis* has only recently expanded to fill its present geographical range.

10-14 The evolutionary origin and genomic organization of SINEs in *Arabidopsis thaliana*

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We have characterized three families of SINE retroposons present in *Arabidopsis thaliana*. The origin, distribution, organization and evolutionary history of RAthE1, RAthE2 and RAthE3 elements were studied and compared to the well characterized SINE S1 element from *Brassica*. Our studies show that RAthE1 and RAthE3 have a common tRNA precursor while RAthE2 and S1 have a different origin. Therefore, these four SINE families have originated from three different tRNAs. The RAthE1, RAthE2 and RAthE3 families are older than the S1 family and are present in all tested *Cruciferae* species. The evolutionary histories of the RAthE1 and the related RAthE3 families are unusual for SINEs. These elements cannot be classified in distinct subfamilies of different evolutionary ages as is the case for S1, RAthE2 and mammalians SINEs. Instead, most RAthE1 and RAthE3 elements were probably derived steadily from two distinct founder sequences that were maintained intact and active for at least 12-20 Myr, a result suggesting that these founder sequences were under selection. The distribution of RAthE1, RAthE2 and RAthE3 elements on the *Arabidopsis* physical map was studied. We observed that, in contrast to other *Arabidopsis* transposable elements, SINEs are not concentrated in heterochromatic regions. Instead, SINEs are grouped in euchromatic chromosome territories several hundred kilobase pairs long. In these territories, SINE elements are closely associated with genes. A retroposition partnership between *Arabidopsis* SINEs and LINEs is proposed.

10-15 Axillary meristem development in the branchless Zu-0 ecotype of *Arabidopsis thaliana*

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Axillary meristems form in the leaf axils during post-embryonic development. In order to initiate the genetic dissection of axillary meristem development, we have characterized the late-flowering branchless ecotype of Arabidopsis, Zu-0. The oldest rosette leaves of Zu-0 plants all initiate axillary meristems, but leaves from the upper part of the rosette remain branchless. Alteration in the meristem development is axillary meristem specific because the shoot apical and floral meristems develop normally. Scanning electron microscopy, histology and RNA in situ hybridization with SHOOTMERISTEMLESS (STM), a marker for meristematic tissues, show that a mound of cells form and STM mRNA accumulates in barren leaf axils, indicating that axillary meristems initiate but arrest in their development prior to organizing a meristem proper. A genetic analysis suggests that the branchless phenotype arises due to a single recessive allele whose effect on the branching pattern of Zu-0 plants can be suppressed by early flowering.

10-16 Analysis of Limburg, an Arabidopsis late-flowering aerial rosette-bearing ecotype

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Flowering time is regulated by a complex genetic network that integrates environmental cues with the developmental state of the plant. To elucidate the molecular mechanism of flowering in Arabidopsis we investigated the genetic basis of aerial rosette formation in late-flowering Arabidopsis ecotypes such as Limburg (Li). Delayed transition to reproductive development in Li plants can be suppressed by vernalization, and the transition to reproductive development can be completely abolished if plants are grown under a short photoperiod. These physiological responses suggest that Li carries genes acting in the autonomous flowering pathway. Genetic analyses indicates that dominant alleles of two loci are responsible for the late -flowering aerial rosette-bearing phenotype. These loci have been identified as new alleles of the late flowering genes *FRI* and *FLC*. There is evidence that an additional genetic factor (*ART3*, *a*erial *r*ose*t*te3) may be required for the extreme late flowering phenotype of Li plants. This evidence includes: the extended linkage between the late-flowering aerial-rosette phenotype to the distal 60cM of chromosome V, and variable flowering behavior of the *fri*-Ler *fri*-Ler *FLC*-Li *FLC*-Li lines (they either flower early, producing ~ 15 leaves, as typical for other *FLC*-containing lines; or they flower after producing * 40 leaves). Results of the experiments aimed at identifying and characterizing the *ART3* locus will be presented.

10-17 Activation of *FLC* by *ART1*, *ART2* and *FRI* is required for the altered body plan of the Sy-0 ecotype of Arabidopsis

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The late-flowering behavior of Arabidopsis winter-annual ecotypes is mainly conferred by two genes, *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*). Sy-0 is a late-flowering Arabidopsis ecotype characterized by an enlarged basal rosette, aerial rosettes and reversion of the inflorescence and floral meristems. Genetic analysis has revealed the requirement of dominant alleles at four loci for the establishment of late flowering in Sy-0. These have been identified as FRI, FLC and two novel flowering loci designated *ART1* and *ART2* (aerial rosette 1 and 2). *ART1* maps 20 cM proximal to *FLC* on chromosome V. The ART1 gene has been localized to a 15kb genomic region and its cloning and molecular characterization are currently underway. Genetic interaction studies show that ART1 and *ART2* cause transcriptional activation of *FLC* independently of *FRI* and *Id*. Thus, *ART1* and *ART2* delineate a novel flowering sub-pathway that interacts with the FRI-activated pathway at or upstream of FLC. These novel interactions among transcriptional regulators of *FLC*, discovered in a naturally occurring ecotype of Arabidopsis, cause a heterochronic shift in shoot apical meristem development that results in a novel Arabidopsis morphology. Therefore, understanding the molecular mechanism of *ART1/ART2* action would allow us to gain an insight into the regulation of flowering in Arabidopsis and would also uncover one of the mechanisms that lead to the evolution of plant morphological diversity.

10-18 Analysis of a functional *CLAVATA3* orthologue from *Capsella rubella*

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Stem cell fate in the shoot and floral meristems of Arabidopsis is controlled by two antagonistic activities. The homeobox gene *WUSCHEL* promotes stem cell fate at the tip of meristems. These cells express *CLV3*, which acts as a ligand to activate the *CLV1/CLV2* receptor complex, resulting in a restriction of *WUS* expression. The analysis of the *FASCIATED EAR2* gene from maize, which encodes a *CLAVATA2* related gene, has indicated that the *CLAVATA* pathway for regulation of meristem size is conserved among the angiosperms. We have now isolated a *CLV3* orthologue (*CrCLV3*) from *Capsella rubella*, a Brassica species that is larger, but morphologically very similar to Arabidopsis, and found that the *CrCLV3* gene can fully rescue a clv3 mutant of Arabidopsis. In situ hybridisation studies show that *CrCLV3*, like *AtCLV3* in Arabidopsis, is expressed in the central zone of Capsella meristems.

10-19 Chromosome homeology between *A. thaliana* and its relatives as revealed by chromosome painting

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After establishing the first chromosome painting for a euploid plant, *A. thaliana* (Lysak et al., 2001, Plant J. 28), we have employed Arabidopsis BAC contigs for comparative chromosome painting to analyse chromosome homeology and karyotype evolution between *A. thaliana* (2n=10) and related species. BAC contigs covering Arabidopsis chromosome 4 (17.5 Mb) were cross-hybridized to chromosome complements of its wild relatives *Capsella rubella* (2n=16), *Cardaminopsis arenosa* (2n=32) and *A. suecica* (2n=26). Four large regions (~1.1 Mb each), homeologous to Arabidopsis chromosome 4, were identified within the chromosome complement of *C. rubella*. Within two subspecies of *C. arenosa* (ssp. *arenosa* and *borbasi*), we found chromosome pairs homeologous to chromosome arms 4S and 4L of Arabidopsis. Preliminary painting data confirmed *A. thaliana* as one and indicated *C. arenosa* as the other parental species of the allotetraploid *A. suecica*. From these data we conclude: i) the high degree of genome colinearity between Arabidopsis and Capsella (Schmidt et al. 2001, Plant Physiol. Biochem. 39) will allow to identify homeologous linkage groups at chromosomal level by comparative painting; ii) *A. thaliana* and *C. arenosa* are closely related; iii) *A. suecica* represents indeed an allotetraploid hybrid between *A. thaliana* and *C. arenosa*; iv) Arabidopsis chromosome 4 originated from a rearrangement involving at least two ancestral chromosomes that might be identical for *C. arenosa* and *C. rubella.*

10-20 QTL analysis of in vitro organogenesis ability in *Arabidopsis thaliana* using two recombinant inbred lines populations

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The *in vitro* regeneration ability variation among the *Arabidopsis thaliana* ecotypes Landsberg *erecta* (Ler), Columbia (Col) and Cape Verde islands (Cvi) previously described for leaf and root explants (Candela *et al* , 2001) have been further analyzed and mapped by using two recombinant inbred lines (RILs) populations. The regeneration ability of 98 RILs Ler x Col (Lister and Dean, 1993) and 162 RILs L*er* x Cvi (Alonso-Blanco *et al* , 1998) was evaluated by two indices: average number of regenerated shoots per callus (SRC) and regeneration rate (RR).

Several loci were identified on chromosomes 1, 2, 4 and 5 using the multiple-QTL-model (MQM) procedure for mapping quantitative trait loci. Some of them have been identified on both kind of RILs indicating coincidences between the regions in which Ler differs from Col and Cvi. However, some regions only appeared when using one or the other RILs type as an evidence of the existence of different Col or Cvi alleles in loci which play a role on the organogenic process.

On the other hand, different chromosomal regions are also detected depending on the kind of explant suggesting the existence of some different phases of the dedifferentiation and redifferentiation processes.

10-21 Identification of *FLM/MAF1* as a quantitative trait locus

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To discover new loci controlling flowering time in natural populations, we have analyzed the flowering time of a recombinant inbred line (RIL) population derived from crosses of Niederzenz (Nd-1) to Columbia (Col-3 and Col-5; Holub, et al., Adv. Bot. Res. 24[1997]). In short days, the 98 RILs show a wide range of flowering times and preliminary QTL analysis using available genotype information indicated the existence of at least two QTL. To gain further mapping power and resolution, we genotyped the RILs for amplified fragment length polymorphisms (AFLPs) using dye-labeled primers. We identified 128 markers, which were then arranged into a genetic map using the 26 previously genotyped markers as anchors. With the new map, we detected four significant QTL: a major QTL, termed *FLOWERING1*, on the bottom of chromosome 1; two QTL on chromosome 2; and one QTL on chromosome 5. *FLOWERING1* maps very close to *FLM/MAF1*, a gene encoding a MADS-domain transcription factor that has recently been shown by the Amasino and Riechmann labs to have flowering time effects in both long and short days. Additionally, the phenotypes of *flm-1 and flm-2*, two T-DNA alleles isolated in the Ws background, agree well with that of *FLOWERING1*. Upon attempting to sequence the *FLM* gene from Nd-1, we discovered a 6.8 kb deletion removing the entire coding region. Out of 140 accessions analyzed, only one additional accession from Niederzenz shared this deletion. Progress on molecular complementation and a quantitative complementation test will be presented.

10-22 The apical hook in *Arabidopsis thaliana* has an adaptive value

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In nature seedling emergence from soil is crucial for completion of the plant life cycle. We have isolated the *Ech* locus from the Arabidopsis accession, C24, exhibiting improved seedling emergence when germinated in soil. When grown in laboratory conditions etiolated *Ech* seedlings, compared with the standard lab accessions, exhibit prolonged maintenance and enhanced curvature of the apical hook. We have studied these traits in a near-isogenic line, *Ech*, containing the C24 locus in Col background. The dominant *Ech* locus specifically affects maintenance of the apical hook. As auxin and ethylene pathways regulate maintenance of curvature we studied both pathways in *Ech* seedlings. Our results suggest that Ech is involved in auxin responsiveness in the apical hook, but does not regulate the ethylene pathway. We found enhanced ethylene response in *Ech* seedlings and suggest that the auxin pathway can modulate ethylene responsiveness during hook maintenance. Differences in emergence rates between *Ech* and *hookless* mutant seedlings suggest that the apical hook is crucial for seedling emergence from soil.

10-23 An analysis of microsatellite variation within *Arabidopsis thaliana*

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In recent years the available natural variation in *Arabidopsis thaliana* has become more widely recognized and utilized along several lines of investigation. Unfortunately, the extensive mapping infrastructure in place for the most common “wildtype” strains of Arabidopsis does not yet extend to the vast majority of natural accessions. Here we present size data and analyses of 20 microsatellite loci for ~130 single seed descent accessions established from natural populations of Arabidopsis. Additionally, we present sequence data for ~20 populations for six microsatellite loci with varying allele size class distributions. Sequence data were gathered with a primary focus on addressing issues of size homoplasy for microsatellite alleles in Arabidopsis and secondarily for examining microsatellite locus stability. Our data indicate that size homoplasy occurs at a relatively high frequency and that this phenomenon is facilitated by indels both within the repeated region and in flanking regions between priming sites and the microsatellite locus. Furthermore, microsatellite stability in Arabidopsis appears to be the result of a balance between the number of repeats at a locus (as has been described for many organisms) and interruptions (insertions) within the repeated segment. The implications of these data in light of microsatellite loci use in mapping and population genetics will be further discussed.

10-24 Responses of 35 ecotypes to five CO_2 atmospheres

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This experiment examined responses to variation in the availability of atmospheric CO2, an essential plant resource. Thus far, we haveassessed the following: 1) genetic variation in responses to growth CO_2 concentration, and 2) identification of the best parental ecotypes for recombinant inbred line populations. 35 standardecotypes of *A. thaliana* from the ABRC collections were subjected to five atmospheric CO_2 concentrations in a balanced factorial design inwhich 16 replicates of each ecotype were grown in each of two replicate chambers at each CO_2 concentration. The following CO_2 concentrations were used: 250, 355, 530, 710 and 900 ppm. We thus spanned the range of CO_2 atmospheres seen on this planet from pre-industrial times to those we expect from continued fossil fuel burning in the coming century. We measured more than thirty traits, including traits associated with developmental timing, carbon assimilation and allocation, nitrogen assimilation and allocation,total mass and its allocation, and reproduction. Multivariate analysis of variance indicated that, for the suite of traitsconsidered together, the ecotypes were highly significantly heterogeneous in their responses to CO_2 level. The greatest mass and reproduction were obtained when plants were grown at 710 ppm, not at the highest CO_2 concentration of 900 ppm. Information will be presented which indicates the best crosses for founding RILs for a variety of traits measured at current CO_2 levels (i.e. forinvestigating the genetic basis of traits under current conditions),and for both pre-industrial and elevated CO2 atmospheres.

10-25 Natural variation in the morphological and chemical characteristics of the stems of Arabidopsis ecotypes

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The chemical composition of plants and the shape of plant fibres are critical to their use as a raw material for the manufacture of pulp and paper. As part of a project aimed at investigating the genetic control of fibre properties, analytical methods have been developed for determining the fibre shape and chemical composition of Arabidopsis. These techniques have been applied to 18 Arabidopsis ecotypes. The cross-sectional dimensions of the fibres in the ecotypes were determined by microscopy of stem sections. Fibre length and coarseness values were determined by pulping the stems followed by analysis using a Fibre Quality Analyzer. The chemical composition of the stems was determined using high performance liquid chromatography and gas chromatography. Length weighted fibre lengths ranged from 0.6 mm for Cape Verde WT-18-02 to 1 mm for Columbia (Col-PRL) WT-01B-02. Fibre coarseness values ranged from 0.062 mg/m for Nossen (No-0) WT-09-03 to 0.140 mg/m for Dijon G WT-10-01. Variations in fibre coarseness' were reflected in differing cell wall thicknesses. Chemical analysis of the stems provided the contents of lignin, the various sugars, the different classes of extractives and inorganics.

11-01 Genetic and epigenetic processes during seed development

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Angiosperm seed development is a result of genetic contribution from the paternal gametophyte, maternal gametophyte and the maternal sporophyte. The development of the seed is thus a developmentally complex process involving the contribution of genes from diverse origin and reproductive strategies.
One important strategy of evolutionary significance is genomic imprinting and the consequent parent of origin effect. Both genetic and epigenetic processes are involved in mediating this effect. Our earlier work with the FIS family of genes indicated that the expression of the FIS genes is under the control of imprinting and that the impaired seed caused by fis mutations can be rescued by reduced methylation of the paternal genome.
A genetic/epigenetic model of seed development will be used to explain these results.

11-02 Sequences of the *FLC* gene required for vernalization response

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Vernalization, the promotion of flowering by a prolonged period of low temperature, is an important control of the time of flowering in plants from temperate regions. Exposure to low temperature usually occurs at the germinating seed or young seedling stage, but the effect of the cold is seen much later in the adult plant. This temporal separation between exposure to and effect of the cold, and the lack of transmission of the effect to progeny, suggests that vernalization has an epigenetic basis. The isolation of *FLOWERING LOCUS C* (*FLC*) has provided insight into the molecular mechanisms involved in vernalization. *FLC* encodes a MADS-box protein which acts as a repressor of flowering, with the level of expression of *FLC* correlating with the time taken to flower. Vernalization promotes flowering by causing a decrease in *FLC* expression. As with the vernalized state, the down-regulation of *FLC* by vernalization is mitotically stable and is reset in progeny, suggesting that the epigenetic regulation of *FLC* is central to the vernalization mechanism. The repression of *FLC* by vernalization has two components: down-regulation during the cold-treatment, and maintenance of the repression after removal from the cold, which requires the product of the *VERNALIZATION2* gene (1). We have used a series of *FLC*::GUS constructs to identify the control regions required for the repression of *FLC* by vernalization, and have shown that distinct regions are required for the initial down-regulation and for the maintenance of the repression.

Gendall et al. (2001) Cell 107, 525

11-03 The epigenetics of heterochromatin

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The Arabidopsis genome is unique among model genomes in having extensivelysequenced heterochromatic regions characterized by both DNA and histonemodification. We have profiled chromatin modification and gene expressionover a heterochromatic portion of chromosome 4 in a variety of mutantsusing microarrays. Transposable elements comprise a large portion ofheterochromatin in Arabidopsis, and we are testing the idea that transposons influence its expression and modification.

11-04 Isolation and characterization of new mutants affected in the establishment of antero-posterior polarity in the endosperm of *Arabidopsis thaliana*

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Our group is interested in endosperm development in *Arabidopsis thaliana*, as a model for early developmental events. The endosperm surrounds the embryo in the seed of flowering plants. An exciting feature during its development is the establishment of an antero-posterior structural and functional polarity: after a syncitial stage, cellularization occurs in the anterior pole, where the embryo grows, whereas a large multinucleated cyst characterizes the posterior pole, which conducts nutrients towards the embryo.
An enhancer-trap line from Jim Haseloff's library, KS117, was characterized in our lab. In this line, the GFP is first expressed in all over the endosperm, and later gets localized to the posterior pole. Thus, we consider KS117 as a good marker for endosperm polarization process. During a screen for mutants affected in endosperm polarization, we isolated 20 lines that develop ectopic posterior multinucleated nodules. The absence of restriction of the KS117 GFP expression confirms their lack of polarization. The genetic control of all these 20 mutations is maternal gametophytic. Some of these mutants are allelic to fis mutants, which our group already described as polarity mutants. FIS proteins are Polycomb group proteins, involved, in drosophila and mammals, in epigenetic controls of transcription domains. Anyway, phenotypic and mapping data let us assume that 2 lines at least are mutated in new loci. Isolating these mutated genes would allow us to further describe the FIS complex components, or the FIS pathway for endosperm development control.

11-05 Arabidopsis requires DNA methylation to maintain heterochromatin organisation

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The higher order organisation of chromatin is essential for correct gene expression. In interphase nuclei of Arabidopsis, chromosomes are arranged as condensed (heterochromatic) chromocentres surrounded by less condensed euchromatin. We have studied the influence of DNA methylation on chromatin organisation by using two mutants (*ddm1* and *met1*) with reduced DNA methylation. Immunolabelling of 5-methylcytosine revealed a dispersed pattern of DNA methylation in *ddm1* and *met1* with weak methylation signals in chromocentres. These appear to be 30% reduced in size compared to wild-type chromocentres. FISH with different Arabidopsis pericentromeric sequences revealed that size reduction of chromocentres correlated with a dispersion of low copy sequences while transposable elements remained at the chromocentres. The positional relationship to chromocentres of rDNA, centromeric repeats and two epigentically-regulated genes (*FWA* and *SUP*) was not altered in mutant nuclei. Within nuclei of backcross progenies, heterozygous for *ddm1* or *met1*, half of the chromocentres showed a wild-type morphology and methylation pattern, whereas the other half was mutant-like. This implies that the presence of *DDM1* and *MET1* gene products cannot restore DNA methylation and heterochromatin structure once it has been lost. We conclude that DNA methylation is an important factor for maintenance of heterochromatin, most likely by triggering heterochromatin-specific histone modifications (see poster Jasencakova et al.).

11-06 Towards genetic dissection of RNA-directed DNA methylation in *Arabidopsis thaliana*

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Homology-dependent gene silencing can occur transcriptionally and post-transcriptionally, affecting either mRNA synthesis in the nucleus or mRNA stability in the cytoplasm. Double stranded RNA (dsRNA) is known to induce a post-transcriptional gene silencing (PTGS) process in the cytoplasm, termed RNA-interference (RNAi), PTGS or quelling in diverse organisms. DsRNA containing promoter sequences, however, triggers transcriptional inactivation of homologous promoters in the genome which is correlated with extensive cytosine methylation – a hallmark of RNA-directed DNA methylation (RdDM). Similar to the double stranded RNA involved in RNAi, this promoter dsRNA which is synthesized in the nucleus, is processed to small RNAs slightly over 20 nucleotides in length. Recently a two-component transgene-based system for studying the process of RdDM was established in *Arabidopsis*: A target nopaline synthase promoter (NOSpro) driving the expression of *NPTII* as a marker gene is efficiently inactivated *in trans* by transcription of a NOSpro inverted repeat resulting in NOSpro dsRNA. Currently, known mutant alleles of three different protein classes are analyzed for their influence on NOSpro silencing and methylation: (1) DNA methyltransferases, (2) chromatin remodelling factors and (3) putative dsRNA processing enzymes. In addition, mutants in PTGS are analyzed in the NOSpro system to identify the extent of overlap between transcriptional and post-transcriptional silencing mechanisms. Finally, the RdDM system was mutagenized both by T-DNA insertion and EMS and screened for reactivation of *NPTII* transcription by kanamycin selection. One mutant (*rts1*; RNA-directed transcriptional silencing), from which two alleles have been recovered so far, still shows considerable NOSpro methylation but almost full reactivation of kanamycin-resistance, thus partially decoupling promoter-methylation from transcriptional reactivation. Furthermore *rts1* maps to a region where no DNA-methyltransferases are annotated. Recent results of genetic analysis of RdDM in Arabidopsis will be presented.

11-07 Maintaining an edge: Antagonistic action of telomerase and the NHEJ DNA repair protein Ku70 at Arabidopsis telomeres

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The nucleoprotein complex at telomeres is essential for genome integrity. In most eukaryotes telomerase is responsible for maintaining the telomeric DNA tract, while non-nucleosomal proteins form the protective cap on the terminus. Among these telomere-associated proteins is the Ku70/80 heterodimer, which is a critical component of the non-homologous end-joining (NHEJ) DNA repair pathway. Our group is exploiting Arabidopsis as a new model system for telomere biology in higher eukaryotes. Recently, we showed that Arabidopsis mutants carrying a T-DNA insertion in the telomerase catalytic subunit, TERT, are viable for up to 10 generations. However, telomeres become much more homogeneous than in wild type and shorten by approximately 500 bp per generation. Progressive telomere erosion ultimately leads to severe cytogenetic damage and developmental arrest (Science (2001) 291:1797). Here we describe the role of Ku70 at plant telomeres. Plants harboring a T-DNA insertion in KU70 are hypersensitive to DNA damage, but in striking contrast to their counterparts in mammals, exhibit no cytogenetic, growth or developmental defects. Unexpectedly, we also found that telomeres in ku70 mutants are much longer and more heterogeneous than wild type (Riha et al., EMBO J, in press). To elucidate the mechanism of telomere lengthening, we generated a ku70/tert double mutant. Remarkably, telomeres in the double mutant closely resemble wild type in length and homogeneity. Thu, Ku behaves as an antagonist of telomerase, and perhaps other activities involved in telomere length homeostasis. Taken together, our findings reveal fundamental differences in plant and animal telomere biology.

11-08 Chromatin organization in (de)differentiating interphase nuclei of Arabidopsis leaf parenchyma cells

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Although somatic cells within a eukaryotic organism carry the same genetic information, their nuclear organization can vary dramatically due to the dynamic nature of heterochromatin and euchromatin. Recently, several papers demonstrated the relationship between epigenetic gene regulation and chromatin organization in yeast, Drosophila and mammalian cells. In contrast, little is known about chromatin remodeling in plant nuclei. Our aim is to get more insight into the epigenetic processes and the chromatin changes during cell (de)differentiation in plants.
We have chosen young rosette leaf tissue of Arabidopsis to study nuclei because of its homogeneity and capability to (de)differentiate. Molecular and immunocytogenetic techniques allow us to visualize the position of chromosomal regions relative to heterochromatin and euchromatin. Previous studies have shown co-localization of (peri)centromeric repeats with methylated chromocenters. In this study we have examined chromatin organization in developing leaf cells and established the nuclear phenotypes using a set of parameters including relative heterochromatin content, DNA and histone methylation patterns and the spatial position of (peri)centrometric and ribosomal repeats.Leaf differentiation appears to be accompanied by an increase in heterochromatin content and number of chromocenters. In addition, there is an increase in DNA methylation at the chromocenters, which suggests silencing of genes during differentiation. In contrast, the same leaf cells show a dramatic reduction in heterochromatin content and chromocenter number upon formation of protoplasts. This suggests a reversion of the nuclear program and possible resetting of silenced genes into a potentially active state. A correlation between differentiation state and chromatin structure will be discussed in the poster.

11-09 Functional analysis of the Arabidopsis *RecQsim* gene

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Arabidopsis thaliana contains seven genes that belong to the *RecQ* family of ATP- dependent DNA helicases. *RecQ* members in *Saccharomyces cerevisiae* (*SGS1*) and human (*WRN, BLM* and *RecQl4*) are involved in DNA recombination, repair and genome stability maintenance, however, little is known about the function of their plant counterparts. In this poster we present the analysis of the Arabidopsis *RecQsim* gene. The *RecQsim* gene is unique among all *RecQ-like* genes that have been isolated from different organisms, due to an insertion of ~100 amino acids inside the helicase domain. We isolated the *AtRecQsim* orthologue from rice and established the presence of a similar insertion. The expression pattern of the *AtRecQsim* gene was compared with the other Arabidopsis *RecQ-like* genes in different tissues and under different stress conditions. Moreover, an *AtRecQsim* T-DNA knockout mutant was isolated and shown to be more sensitive to DNA damaging agents as compared to the wild type. The yeast *sgs1* mutant, which shows hypersensitivity to DNA damaging agents, including methyl-methanesulfonate (MMS), was transformed with the *AtRecQsim* cDNA. Our results show that the *AtRecQsim* gene can suppress the MMS sensitivity phenotype of the yeast *sgs1* mutant. These results, together, suggest that the *AtRecQsim* gene, though unique in structure for plants, is involved in DNA repair.

11-10 Characterization of carbon ion-induced mutations in *Arabidopsis thaliana*

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Irradiation of *Arabidopsis thaliana* by carbon ions was carried out to investigate the mutational effect of ion particles in higher plants. The averaged mutation rate of carbon ions was 2.0 X 10-6/ Gy, which was 17-fold higher than that of electrons. During the mutant screen, two novel *tt* mutant lines (*tt18*, *tt19*) were isolated. PCR analysis of the mutants showed that out of 30 mutant alleles, 14 had point-like mutations within the gene, while 16 contained large structural alterations. Further sequence analysis revealed that most of the point-like mutations were short deletions. To elucidate the nature of structural alterations, three carbon ion-induced mutations, *gl1-3*, *tt4*(C1), and *ttg1-21*, were analyzed in detail. The *gl1-3* mutation was an inversion that involved *GL1* and *Atpk7* loci on chromosome 3. The inversion was found to accompany an insertion of a 107-bp fragment derived from chromosome 2. The *tt4*(C1) mutation was also found to be an inversion. In the case of *ttg1-21*, it was found that a break occurred at the *TTG1* locus on chromosome 5, and a reciprocal translocation took place between chromosome 3. From the sequences flanking the breakpoints, DNA strand breaks were found to be rejoined using, if present, short homologous sequences. Small deletions were also observed around the breakpoints. These results suggest that the non-homologous end joining (NHEJ) pathway operates after plant cells are exposed to ion particles. The isolation of novel mutants and the high mutation rate suggest that ion particle is a valuable mutagen in plant genetics.

11-11 Functional analysis of proteins involved in the regulation of flavonoid biosynthesis in *Arabidopsis thaliana* seeds

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During Arabidopsis seed development, flavonoid compounds accumulate specifically as flavonols and proanthocyanidins in the seed coat (endothelium) to protect the embryo against oxidative stress. Our laboratory focuses on the genetic regulation of the flavonoid pathway with the study of *transparent testa* (*tt*) mutants. We have previously characterised at the molecular level two loci involved in this metabolism regulation: *TT2* and *TT8* which encode an R2R3 MYB DNA binding domain protein and a basic helix-loop-helix factor, respectively (Nesi *et al.*, 2000 and 2001). These two proteins regulate the expression of at least two flavonoid late biosynthetic genes: *BANYULS* (*BAN*) and the *DIHYDROFLAVONOL-REDUCTASE* (*DFR*). A third protein, TTG1, which belongs to the WD40 protein family, is also required for the regulation of *BAN and DFR* expression. These results suggest a possible interaction of several loci in regulating the late biosynthetic steps of the flavonoid pathway in seeds. In order to precise the mode of action of TT2, TT8 and TTG1, we chose to carry out functional analyses in yeast, which is a versatile tool to study transcription factors. We performed two-hybrid analyses to dissect the possible interactions between TT2, TT8 and TTG1 as well as one-hybrid studies to investigate the possible interactions between any of these factors and the *BAN* promotor. Finally, we propose to complete this work with in vitro studies of immunoprecipitation and Electrophoretic Mobility Shift Assay (EMSA).

11-12 5S rDNA transcription in *Arabidopsis thaliana*

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In *Arabidopsis* (Columbia), about 1000 copies of 5S rDNA genes are organized in tandem arrays localized in heterochromatic pericentromeric regions of chromosomes 3, 4 and 5. Each 5S unit (~ 500 bp) contains a 120 bp-transcribed region and a non-transcribed spacer. Analysis of the DNA sequence allowed the definition of specific signatures (T-stretch) and specific restriction polymorphism for each 5S-rDNA array. Analysis of 5S RNA from different tissues has revealed a major 120 bases 5S RNA and some minor 5S RNA varying from the major one by one or two bases substitutions. By using a comparison of the 5S DNA and RNA sequences, we could show that both major and minor 5S RNA come from only two of the genomic 5S loci: chromosome 4 and chromosome 5 major block. The other 5S loci are either not transcribed or produce rapidly degraded 5S transcripts.
We performed genomic sequencing of methylation patterns in 5S-rDNA by using sodium bisulfite mutagenesis. This analysis revealed that every cytosine position within the 5S sequence is highly methylated whatever the context: CpG, CpNpG or non-symmetrical, and whatever the 5S block. The methylation pattern of both transcribed and non-transcribed 5S units is similar, with no preferential methylated or unmethylated site. These results, together with *in vitro* transcription of methylated 5S genes, demonstrate that 5S transcription is not inhibited by methylation. We are now investigating 5S-rDNA genes expression during plant development and in the *ddm1* background. The influence of chromatin structure on 5S expression is also under investigation.

11-13 Histone modifications and heterochromatin assembly in *Arabidopsis thaliana*

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We tested the nuclear distribution of posttranslationally modified histones in Arabidopsis in comparison with that of other plants (Jasencakova et al. 2000; 2001). In Arabidopsis, euchromatin was found to be enriched in acetylated histones H3 and H4, as well as in H3 methylated at K4. Nucleoli and heterochromatic chromocenters were mostly depleted of these modifications. Methylation of K9 of H3 was consistently strong at chromocenters and much weaker at euchromatin and nucleoli. Only K16 (but not K5 and K12) of H4 showed a cell cycle-dependent variation of acetylation intensity at eu- and heterochromatin, while nucleoli revealed no H4Ac16 throughout interphase. Also H3 (in particular K18) revealed a cell cycle and ploidy-dependent intensity of acetylation at heterochromatin and additionally at nucleoli.
Within nuclei of the mutants ddm1 and met1 with severely reduced DNA methylation and decreased heterochromatin content, the heterochromatin-specific histone modifications were restricted to the diminished chromocenters. Large proportions of the pericentromeric heterochromatin became dispersed from the chromocenters, lost its heterochromatic character and acquired euchromatin features, i.e., less dense chromatin structure, elevated acetylation of H3 and H4 and methylation of K4 of H3 as well as reduced methylation of K9 of H3. In summary, i) the nuclear patterns of histone acetylation and of methylation of K9 of H3 may differ between Arabidopsis and plants with larger genomes and more complex chromatin organization; ii) DNA methylation apparently triggers deacetylation of K5, K8 and K12 of H4, demethylation of K4 and methylation of K9 of H3 during heterochromatin assembly in Arabidopsis.

11-14 Functional analysis of transcription factors expressed during seed development in Arabidopsis

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Transcription activating factors (TAF) regulate the expression of target genes through the integration of internal and external signals Although recent data suggest that a number of TAF are implicated in a control-network of gene expression during development, few of them have been characterised at a functional level.
The aim of the project is to identify the function of TAF expressed during seed maturation, either by screening for mutants or by modifying their expressing them in transgenic plants. The identification of associated phenotype(s) as well as the analysis of their expression pattern will allow the identification of target genes involved in the processes controlled by those TAF. Finally, we will try to modify developmental pathways of reserve accumulation during seed development by acting on these regulators.
To date, 150 TAF have been screened for expression in developing siliques, allowing to demonstrate mRNA accumulation for 50 of them expressed in this organ. Some exhibit a pleiotropic expression among organs but others are specifically expressed during silique development. Mutants for 30 of the TAF expressed in the silique have been identified by reverse genetics and the impact of the mutation on the physiology of the developing seed is under investigation. On the same line, promoter sequences of some TAF have been cloned upstream a reporter gene to allow precise identification of their expression pattern.

11-15 Novel DNA gyrase activity from the eukaryotic model plant *Arabidopsis thaliana*

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DNA gyrase is the bacterial enzyme that supercoils DNA using the free energy from ATP hydrolysis. It is a DNA topoisomerase of which there are two types, I and II, distinguished by whether they transiently break one or both strands of the DNA. Gyrase is a type II enzyme that is unique to prokaryotes and essential for the processes of replication and transcription, and is the target of a number of anti-bacterial agents, including quinolones (e.g. ciprofloxacin) and coumarins (e.g. coumermycin). In higher plants, evidence has accumulated supporting plastid replication being analogous to that in prokaryotes. As a circular, double-stranded DNA molecule, the plastid genome raises topological problems for replication. The publication of the complete genome sequence of *Arabidopsis thaliana* revealed the presence of several genes with homology to bacterial *gyrA and gyrB*. The enzyme subunits are nuclear-encoded and targeted to both chloroplasts and mitochondria with N-terminal transit peptides. We germinated *A. thaliana* ecotype Columbia seeds and have grown seedlings at a range of ciprofloxacin concentrations, and found inhibition of seed germination and etiolation from the base of the petiole of 6 week old plants, even at the lowest drug concentration used (1 uM). These effects cannot be reversed by the subsequent removal of drug from the media. Treatment of seedlings in liquid culture with coumermycin severely inhibited growth. Chloroplast morphology in suspension cell cultures is markedly altered in the presence of clinically relevant concentrations of ciprofloxacin. Supercoiling activity has been isolated from extracts of purified chloroplast and mitochondria of the *A. thaliana* cells and purified by affinity chromotography. The activity is ATP-dependent and sensitive to both quinolone and coumarin antibiotics. This represents the first corroborated report of a gyrase in eukaryotes.

11-16 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. A seed-specific promoter (the promoter, which drives expression of the gene encoding the subunit of the conglycinin protein in soybean) was chosen as a target promoter. Transgenic target lines were established for two different reporter genes (GUS and GFP), which were fused to the promoter. Appropriate homozygous target lines were supertransformed with a silencer construct designed to transcribe an inverted repeat of the target promoter by the 35S promoter (constitutive promoter). Dramatic reductions of reporter gene activity were observed in target-silencer double transformant lines. EMS mutagenesis was done against the silenced line from this system and 59 candidate M2 lines were obtained. Characterization of data from several of these candidate lines will be presented.

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

11-17 Formation of stable epialleles and their paramutation-like interaction in tetraploid Arabidopsis

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Several independent tetraploid *Arabidopsis thaliana* lines, derived from the same diploid strain uniformly expressing a transgenic locus over many generations, showed incidents of transcriptional silencing of the transgene. These silent states are stably inherited to tetraploid progeny and also to diploid derivatives obtained after backcrosses to a wild type strain. The loss of expression after polyploidization appears to be due to epigenetic modification accompanied by hypermethylation of the silent loci. Interestingly, new transcriptionally inactive epialleles reduce the expression of an active allele if combined in the same tetraploid genome. This effect shows different degrees of penetrance. Moreover, genetic segregation data indicate that the suppressive effect is lasting even after genetic separation from the silencing allele, thus resembling paramutation. Analogous epialleles do not interact in a diploid background. The frequent occurrence of polyploidy might therefore have contributed to the epigenetic variability and evolution in plants.

11-18 Search for *cis* regulatory elements of genomic imprinting at the Arabidopsis *MEDEA* locus

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The *Arabidopsis medea* (*mea*) mutant exhibits a female gametophytic maternal effect. Seeds that inherit a mutant allele from the female gamete abort irrespective of the paternal contribution. It has been shown previously that this maternal effect is due to the regulation of *MEA* by genomic imprinting (Vielle-Calzada *et al.*, 1999; Kinoshita *et al*., 1999). Genomic imprinting refers to a form of epigenetic regulation whereby imprinted genes show differential expression depending on the sex of the parent from which they are inherited. At the *MEA* locus, only the maternally inherited allele is zygotically transcribed whereas the paternally derived *MEA* copy is silent. The mechanism that controls imprinting at *MEA* is not yet understood. The *Arabidopsis DECREASE IN DNA METHYLATION 1* (*DDM1*) gene encodes a SWI2/SNF2 type chromatin remodelling factor and decreases DNA methylation genome-wide by about 70%. *DDM1* has been shown to be required for the maintenance of the imprint at the *MEA* locus. Seeds that maternally inherit a mutant *mea* copy and paternally inherit a wild type copy of *MEA* are rescued when they are homozygous for the *ddm1* mutation. The actual role of DNA methylation and/or chromatin structure for the epigenetic mark at *MEA* is yet unknown.
We are currently characterizing the methylation profile at the *MEA* locus and are testing regions that are differentially methylated in a *ddm1* mutant background for their relevance to the imprinting mechanism. In addition, we are generating *MEA* promoter deletion constructs fused to the GUS reporter gene in order to define the regions crucial for parent-of-origin dependent *MEA* gene expression.

11-19 Towards biochemical purification of SPLAYED

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Recently, a novel component, with homology to the SWI/SNF family of ATPases, involved in control of reproductive development in *Arabidopsis thaliana* was identified. In wildtype plants, this component, called SPLAYED (SYD), acts as a repressor of the floral transition presumably by being involved in transcriptional regulation. It was found that in SYD mutant plants there were no changes in the levels of transcriptional activators important for floral transition. To understand how SYD acts in development, the gene was cloned, and found to have an ATPase domain characteristic of the SWI/SNF family of ATPases, specifically with high homology to members of the Snf2p subgroup. These proteins are central subunits of multiprotein complexes involved in regulation of transcription via chromatin remodeling. In addition to the ATPase domain, it was also found that SYD has a nuclear localization signal and domains that may mediate tethering to chromatin. From these data it seems plausible that SYD alters the effectiveness of endogenously activated transcription factors through chromatin remodeling. The first steps in characterizing this important developmental regulator have been the focus of this project. An anti-SYD antibody has been generated and biochemical purification of the SYD complex has been initiated.

11-20 Frequency and position of meiotic and mitotic unequal cross-over events within a synthetic *RBCSB* gene cluster

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Paralogous genes that comprise a multigene family are usually dispersed throughout the genome and therefore evolve independently. In contrast, paralogous genes positioned as a linked tandem array can undergo concerted evolution through a process of unequal crossing-over. This will lead to four concomitant changes in the gene cluster: gene duplication(s), gene deletion(s), and two reciprocal recombinant genes. Previously, we developed a synthetic *RBCSB* gene cluster (*synthRBCSB1*) that utilizes the activation of a firefly luciferase gene to identify and isolate chimeric genes and linked gene duplications caused by meiotic unequal crossing over (Jelesko, J, et al, 1999, *PNAS USA*,96:10302-07). To determine the rate of meiotic unequal crossing-over between the *RBCS1B* and *RBCS3B* genes, the *synthRBCSB1-1* transgenic line was crossed to several different Arabidopsis lines. The observed rate of recombination between these two genes ranged from one to four recombinants per one million F2 seedlings. Similar meiotic recombination rates were observed for an independent transformant, *synthRBCSB1-2*. Interestingly, mapping of the meiotic recombination resolution break points showed a non-random distribution recombination resolution break points. To assay rates of mitotic recombination associated with T-DNA insertion, we assayed the frequency of a derivative *synthRBCSB2* gene cluster comprised of a *delta-RBCSB1B::LUC* and *RBCS3B* genes. The frequency of luc+ recombinants due to mitotic recombination was 7.9 x 10-4. The mapping of mitotic recombination break points showed both similarities and significant differences in the positioning of mitotic recombination breakpoints relative to those observed during meiosis.

11-21 Epigenetic regulation of the *FWA* gene during early plant development

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The *FWA* gene, which encodes a homeodomain protein, has been identified through genetic characterization of late flowering *fwa* mutants. Unique feature of the *fwa* mutations is that they are gain-of-function epigenetic alleles. Although nucleotide sequences of the FWA region in the mutant alleles are identical to that in the wild type allele, the mutant alleles are hypomethylated and ectopically expressed. Heritable epigenetic late flowering alleles of the *FWA* gene were also induced in trans by *ddm1* (*decrease in DNA methylation*) mutation. In the wild type plant, no *FWA* transcript has been detected except in the siliques and the imbibed seeds (Soppe et al. 2000, Mol. Cell 6: 791-802).

Although over-expression of *FWA* gene induces delay in flowering onset, its role in normal development remains unanswered. To understand epigenetic regulation and role of the *FWA* gene during normal development, we are focusing on its regulation in the wild type plant. We investigated mRNA distribution by in-situ hybridization and RT-PCR analysis of the dissected seed tissues. Both of the results indicated that *FWA* is specifically expressed in the endosperm. We further examined FWA expression using transgenic lines harboring the *pFWA::FWA-GFP* transgene. The fusion protein was localized to the nucleus of unfertilized mature central cell and the nuclei of the endosperm after fertilization. The epigenetic controlling mechanisms of *FWA* will be discussed.

11-22 Arabidopsis *TERMINAL FLOWER2* (*TFL2*) gene encodes an HP1-like protein and negatively regulates *FT* expression

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Flowering of plants is regulated by many environmental stimuli such as day length and temperature. Mutations in the *TERMINAL FLOWER2* (*TFL2*) gene cause an early-flowering phenotype and strongly reduced photoperiod sensitivity as well as several pleiotropic phenotypes. Among four genes which play key roles in the flowering pathway *(CO, FT, LFY, and SOC1*), only *FT* transcript is highly upregulated in *tfl2* mutants from the early stage of vegetative development. The fact that *ft* mutation suppresses the early flowering phenotype of *tfl2* suggests upregulation of *FT* is the main cause of the early flowering of *tfl2*. We have cloned the *TFL2* gene using a newly isolated T-DNA tagged allele (*tfl2-3*) and found that it encodes a nuclear protein with chromo and chromo-shadow domains. TFL2 has homology with heterochromatin protein1 (HP1), which is known to mediate heterochromatin formation and transcriptional repression of several euchromatic genes. *TFL2* is expressed in undifferentiated cells of shoot apical meristems, floral meristems, young leaves, and root meristems. Transgenic plants expressing TFL2:GFP fusion protein show subnuclear localization of TFL2 in foci. Together, these results suggest that TFL2 functions as an HP1-like epigenetic repressor and regulates flowering time by repressing *FT* expression during non-inductive conditions. We will present the effect of *tfl2* mutation on DNA methylation in the *FT* locus. Also, we are currently looking for the regulatory sequences of *FT* which are involved in the repression by TFL2.

11-23 Inheritance of epigenetic traits induced by *ddm (decrease in DNA methylation)* mutations

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In contrast to mammalian epigenetic phenomena, where reprogramming of gene expression generally occurs in each generation, epigenetic states of plant genes are often inherited over generations. Loss of epigenetic silencing of repeated sequences is induced by mutation in *DDM1* gene, which encodes a protein similar to the chromatin remodeling factor SWI2/SNF2 (Jeddeloh et al., Nature Genet. 22, 94-). A striking feature of the *ddm1* mutation is that it induces a variety of developmental abnormalities by mobilizing transposons (Miura et al., Nature 411, 212-) and by affecting host gene expression (Soppe et al., Mol. Cell 6, 791-, Stokes et al., Genes Dev. 16, 171-). By characterizing out-crossed progeny from the *ddm1* mutants, we examined stability of the late-flowering epigenetic traits and epigenetically-activated transposons in the wild type background. Results on inheritance of DNA methylation pattern and their activities will be presented.

11-24 Plants are different: An archaebacterial topoisomerase homologue not present in other eukaryotes is indispensable for viability of Arabidopsis

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In general all eukaryotes contain in their genome similar sets of homologues involved in basic aspects of DNA metabolism as DNA replication, repair and recombination. Therefore it was very surprising to find that in contrast to other eukaryotes Arabidopsis, and as it seems plants in general, contain a homologue of the B subunit of the archaebacterial topoisomerase VI (AtTopo6B) that is strongly expressed in somatic tissue. The protein is able to interact with two of the three homologues of the A subunit of the topoisomerase present in the Arabidopsis genome (AtSpo11-2 and AtSpo11-3) and is targeted to the plant nucleus. We isolated an Arabidopsis T-DNA insertion mutant of AtTop6B and characterized its phenotype to elucidate the role of the protein in vivo. Mutant plants have a severe growth defect. After the development of a few small leaves the plants stop to grow but die only several weeks later. Electron-microscopic studies indicate a reduced starch and lipid catabolism in the mutant embryonic seedlings, most probably due to reduced growth rate. The number of mitotic figures in the root meristem - an indicator for cell divisions - of the mutant as compared to the wild type was determined to be reduced by half in repeated experiments. Comet assays indicate that the nuclear DNA of the mutant contains more breaks than wild type. These results indicate that AtTopo6B is involved in a basic process of DNA metabolism, the disturbance of which results in a reduced number of cell divisions.

11-25 Domain characterization of an Arabidopsis homolog to Drosophila *BRAHMA*

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During *Drosophila* development the antithetic action of Polycomb and Trithorax group of proteins is responsible for the maintenance of transcriptional states of homeotic genes. Thus, the Trithorax group protein Brahma was identified as a suppressor of polycomb mutations. *brahma* encodes a DNA-dependent ATPase of the SNF2 family. *Arabidopsis* Polycomb homologs with important roles in development have been described during the last years raising the possibility that also in plants a Polycomb-Trithorax interplay controls development. Two closely related *brahma* homologs can be found in the *Arabidopsis* genome: *SPLAYED* and At2g46020, which we named AtBRM. SPLAYED has been previously shown to control flower development (Wagner and Meyerowitz, Curr Biol 2002.12:85-94). We are characterizing the role of AtBRM in chromatin and development. As SPLAYED, AtBRM is mostly expressed in flowers. AtBRM presents an extensive DNA binding domain where we have identified three AT hooks. This region is phosphorylated, in vitro by CDC2-cycB, suggesting that AtBRM may be regulated by phosphorylation during mitosis. AtBRM also contains a bromodomain whose capacity to interact with histones is being currently analyzed.

11-26 The role of genomic imprinting in hybridisation barriers between Arabidopsis species

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Plants have various barriers that minimise gene flow between species. However successful pollen transfer and fertilisation is possible between some species and can result in seed development and even the production of interspecific hybrids. Our work concentrates on the post-fertilisation barrier that exists between *Arabidopsis thaliana* and some of its close relatives

Cross pollination between diploid *A. thaliana* and its close relative, *Arabidopsis arenosa*, produces aborted interspecific seed. However, by artificially doubling the ploidy of the maternal parent, *A. thaliana*, to tetraploid, fertile interspecific seed can be generated which germinates to produce a hybrid, synthetic *Arabidopsis suecica*. The behaviour of the endosperm in these hybrid seed suggests rescue was achieved by restoring a viable balance of maternal and paternal genomes. Genomic imprinting has been proposed to explain endosperm phenotypes and seed viability in reciprocal interploidy crosses in *A. thaliana*. Here we present evidence that suggests the same mechanism underlies the outcome of seed development in *A. thaliana* x *A. arenosa* crosses. Furthermore manipulation of imprinted gene expression by demethylation introduces a hybridisation barrier between tetraploid *A. thaliana* and *A. arenosa*. Introduction of barriers of this kind may potentially prove useful in reducing the spread of GM material from crops to wild relatives. Utilisation of the same mechanism may also render it possible to produce novel hybrids by breaking down existing natural hybridisation barriers.

11-27 DNA ligase IV and ligase interacting factors in DNA double strand break repair in Arabidopsis

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Double strand breaks in DNA can occur spontaneously in the cell or be induced experimentally by gamma-irradiation and represent one of the most serious threats to genomic integrity. Non-homologous end joining (NHEJ) rather than homologous recombination appears to be the major pathway for DSB repair in humans and plants. Biochemical and molecular evidence in yeast and mammals has shown that DNA ligase IV is required for the ligation step in NHEJ. We have recently characterised DNA ligase IV from *Arabidopsis*, and current studies are investigating the role of DNA ligase IV and interacting proteins in NHEJ through biochemical assays, reverse genetics and reporter gene expression. We have also cloned and characterised *Ku70* and *Ku80* from *Arabidopsis* and demonstrated that these proteins form a heterodimer with DNA end binding activity. A *ku80* Arabidopsis knockout mutant shows hypersensitivity to DNA damaging agents consistent with the role of *Ku80* in the repair of DSBs in *Arabidopsis*. These results indicate that the highly conserved NHEJ pathway of DNA double strand break repair is active in *Arabidopsis* and is required for resistance to genotoxic agents.

11-28 Implications of *FAS* and *ASF1* in maintaining the cellular organization of apical meristems

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Chromatin Assembly Factor-1 (CAF-1), a histone chaperon complex, assembles nucleosome preferentially onto newly replicated DNA. FASCIATA1 (FAS1) and FAS2 gene products are functional homologues of CAF-1 p150 and p60 subunit. In fas mutants, cellular organization of SAM and RAM are disturbed, and expressions of WUSCHEL in SAM and SCARECROW in RAM are variegated. Since CAF-1 facilitates rapid formation of chromatin after the passage of replication fork, we assume that stable maintenance of gene expressions are disturbed in proliferating cells of fas mutants.
Anti-Silencing Function 1 (ASF1), another histone binding protein, is known to stimulate CAF-1-dependent nucleosome assembly in vitro. In budding yeast, asf1 mutants showed synergistic defects in gene silencing if crossed with caf1 mutants, suggesting their functional interaction. Actually, ASF1 can bind to CAF-1 p60 subunit. We recently identified two candidates of ASF1 homologues in Arabidopsis, ASF1a and ASF1b, we obtained their mutants from T-DNA insertion populations. We will discuss physiological implications of ASF1 by showing show morphological phenotype and other observations found in asf1a, asf1b, asf1a;asf1b, and/or asf1;fas mutants.

11-29 Splicing factor AtRSZ33 is involved in regulation of cell shape in Arabidopsis

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Splicing provides an additional level in the regulation of gene expression and contributes to the increase of diversity of proteins. We have cloned a new Arabidopsis gene termed atRSZ33, which encodes a protein with an RNA-binding motif, two zinc fingers, arginine/serine (RS) domain and a C-terminal tail rich in serine/proline dipeptides. This protein structure together with results of yeast two-hybrid screening implies that this protein is one of the SR splicing factors, a protein family shown to be involved in constitutive and alternative splicing. Interestingly, atRSZ33 is also subjected to the regulation by alternative splicing; an additional alternative RNA form originating from alternative splicing in the second intron is produced at early stages of seedling development. Overexpression of atRSZ33 led to the production of two more alternative RNA species. These RNA forms of atRSZ33 encode truncated proteins of unknown function. Investigation of an atRSZ33 promoter-GUS fusion and in situ hybridization patterns demonstrated its expression during embryogenesis, in shoot apical and root meristems, in tapetum and mature pollen. Interestingly, transgenic Arabidopsis plants with increased atRSZ33 protein levels showed severe growth phenotype affecting all stages of plant development. Increased cell expansion and changed polarization of cell elongation and division were the main features of all phenotypic changes. Splicing patterns of several Arabidopsis genes were modified by overexpression of atRSZ33.

11-30 Photoreactivation and base excision pathways in *Arabidopsis thaliana*

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The integrity of the plant nuclear and organelle genomes must constantly be maintained in response to DNA damage. We present a characterisation of two contrasting mechanisms for repair of base damage in *Arabidopsis thaliana*. CPD (cyclobutane pyrimidine dimer) and 6-4 PPD (pyrimidine 6-4 pyrimidone) specific DNA photolyases mediate the light-dependent repair (photoreactivation) of UV-B induced damage products by error-free direct reversal of base damage. Unlike the constitutively expressed 6-4PP photolyase, white light and UV-B induce and regulate CPD photolyase expression in Arabidopsis. Studies indicate that Arabidopsis possesses the photorepair capacity to respond effectively to predicted increases in UV-B irradiation and global warming in the twenty-first century.

In contrast to photoreactivation, excision repair pathways (dark repair) involve several enzyme mediated steps. The *Arabidopsis* genome sequence contains putative orthologues of mammalian and yeast excision repair proteins, including a uracil DNA glycosylase (UDG). Uracil may arise in DNA through the misincorporation of dUMP during replication or oxidative deamination of cytosine to uracil, the latter being a major cause of mutagenesis in DNA if unrepaired. Removal of uracil from DNA is effected by base excision repair (BER) pathways, with (UDG) as the first enzyme in this pathway. Unlike animals, which have two distinct UDG activities (SMUG1; UDG), *Arabidopsis* has only a single UDG gene. UDG is constitutively expressed in both *Arabidopsis* and rice. The results of UDG characterisation *in vitro* using the purified recombinant protein and *in vivo* using GUS and GFP reporter gene expression will be presented.

11-31 Role of the Ku80 protein in telomere maintenance in *Arabidopsis thaliana*

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Telomeres are specialized DNA/protein structures that allow cells to distinguish chromosome ends from double-strand breaks and so preventing chromosome fusion events. At present it is not clear which particular composant(s) of the telomere structure are essential to protect chromosome ends from DNA repair activities. Interestingly, several proteins identified by their important roles in DNA repair have recently been shown to play additional roles in telomere maintenance. The complex Rad50-Mre11-Xrs2 and the Ku heterodimer have been shown in yeast to be involved in telomere regulation. Recently the role of the Ku protein at telomeres has been demonstrated in mammalian cells. We have reported that Rad50 protein is essential for telomere maintenance in *Arabidopsis thaliana*. Here we show the characterization of an *Arabidopsis thaliana* plant containing a T-DNA insertion in the AtKu80 gene. We have measured telomere length in wild-type and Ku80 deficient Arabidopsis plants and cells. We have observed that Ku deficiency results in lengthening of telomeres. This is in contrast with results from yeast and mammals. Ku-mutants in yeast result in telomere shortening while no clear effect on telomere length is observed in Ku deficient mammalian cells. Our results show for the first time the implication of the Ku protein in telomere regulation in plant cells.

11-32 A novel forward genetic system for the efficient isolation of genes involved in the maintenance of transcriptional gene silencing

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Transcriptional gene silencing (TGS) can inactivate transgene expression in plants by suppressing transcriptional initiation. Some of the components participating in TGS were identified in genetic screens for mutants in *Arabidopsis* that reactivate transcriptionally silenced transgenes. However, these screens may not be exhaustive due to the limitations of custom-made mutagenesis in the background of transgenic lines, due to the requirement for a selection system or to the instability of silencing. Recently, endogenous targets of TGS termed TSI (transcriptionally silent information) were identified (Steimer et al., Plant Cell 12: 1165-1178 (2000)). TSI is expressed as non-coding RNA in all well-characterized TGS, and therefore TSI expression appears to be an ideal tool to detect novel mutants defective in TGS maintenance. Using a RT-PCR based approach we established a new screening system that was independent of the transgenic background and did not require selection to identify additional TGS mutants in large mutant pools. We demonstrated that we could amplify TSI if mom1 tissue was diluted a thousand-fold with Wild type tissue that did not express TSI, indicating the sensitivity of the screen. To date we have screened RNA pools extracted from 12000 T-DNA-tagged *Arabidopsis* lines of the Columbia ecotype. We identified 11 independent mutants in which TSI expression was confirmed by Northern analysis inferring that the designed screen is an efficient method by which to identify mutations in TGS.

11-33 Identification of an *emb* mutant indicates role of *SET*-Domain genes early in the embryo development in Arabidosis

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Hundreds of mutants belonging to the embryo-lethal or embryo-defective (*emb*) group have been identified and the estimated number of *EMB*-genes is close to 500. An Arabidopsis-line (line 113) with a T-DNA insertion in a *SET*-domain gene was identified in our lab. SET-domain proteins are thought to be involved in chromatin remodelling. Plants of this line show a normal phenotype, except that approximately 1/4 of the embryos in each silique are of the raspberry-type. The phenotype seems to be caused by a homozygous recessive mutation. Microscopy of the seeds showed a defect both in the embryo and suspensor region and lack of a clear hypophysis. The mutant appears to be arrested after the first proembryo stage and seems to be homozygous-lethal. Mutant seeds fail to give seedlings. Out-crossing of the mutant with wt plants and subsequent selfing for three generations have shown that the mutant seeds always resides in siliques of plants hemizygous for the T-DNA insertion, monitored by Km selection. Hence, the T-DNA is closely linked to the mutation. The mutant is different from the previous described emb mutants and we are presently studying the wild type expression pattern of the tagged *SET*-domain gene. Consistent with the early embryo mutant phenotype, RT-PCR on mRNA from wt (C24) detects the transcript in buds and flowers but not in mature seeds. Other methods will be used to investigate the expression pattern in more detail. Currently we are working on further analysis of the mutant including complementation of the mutant phenotype.

11-34 News on homologous recombination, chromatin remodelling and mutants

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Homologous recombination is an important process, both in meiosis and during somatic development. Strikingly, this process is involved both in genome flexibility, which is important for evolution, and genome stability by participating in DNA repair process. 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. We wanted to identify plant components involved in regulatory pathways and in recombination process itself.
Using homologous recombination as a marker for genome dynamics, we established and screened a mutant collection of *Arabidopsis thaliana* ecotype Columbia for altered recombination phenotypes in the absence of external stress. In order to monitor recombination directly *in vivo* we used a luciferase reporter line designed for detection of intrachromosomal recombination by restoration of functional luciferase transgene. 20 000 independent activation-tagging transformants
from this line were directly screened for a dominant effect. This gave rise to 30 candidates with an increase in the number of luciferase sectors ranging from 10 to 100 fold. One of these candidates was mutated in a putative SWI/SNF ATPase. The effect of the mutation (Knock-Out, antisense) on recombination has been assessed.
The genetic characterisation of the mutant and the molecular analysis of the putatively affected genes are presented. These results might provide new powerful insights into the regulation of genome integrity.

11-35 A mutant plant with increased frequency in homologous recombination

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Homologous recombination plays a dual role in many organisms: its involvement in DNA repair ensures the maintenance of genome integrity whereas meiotic cross-over allows genome flexibility. Unlike other systems, plant cells only rarely use the homologous recombination pathway for repair of DNA damage, thereby avoiding targeted genetic modifications of plants.
Biotic and abiotic environmental factors are known to alter the frequency of somatic recombination events, but in plants there is poor knowledge about genes engaged in sensing these factors, in signaling and in execution of the recombination process. Genetic screens should reveal components involved either directly in the repair process or indirectly in chromatin modeling, in DNA damage signaling or in cell cycle control.
Here, we present a Arabidopsis mutant with increased somatic homologous recombination frequency, which was isolated from a T-DNA mutagenized population harboring a transgene-based substrate to visualize recombination events. The mutagenizing T-DNA was altering a locus with a cluster of genes potentially involved in DNA metabolism. Since any of these genes could possibly influence genome integrity, we are currently investigating the cause of the upregulated homologous recombination phenotype.

11-36 High, stable and uniform expression of transgenes in *Arabidopsis thaliana*

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Predictable and stable transgene expression is a prerequisite for the broad use of transgenic plants. But in populations of transgenic plants variability of transgene expression over several orders of magnitude and even gene silencing is frequently observed.
For a comprehensive study of transgene expression in *A. thaliana*, we established lines with a single-copy of a T-DNA harbouring a reporter gene under the control of the CaMV 35S promoter. We used the chimaeric ß-glucuronidase gene, the streptomycin-phosphotransferase gene and the gene for the green fluorescent protein as reporters. The expression of the transgenes is high, stable over all generations analysed and comparable between independent lines containing two copies of a particular transgene. Most importantly, characterisation of more than 70 independent single-copy T-DNA transformants revealed no case of silencing due to site of T-DNA integration within the genome.
Lines carrying the T-DNA in a hemizygous fashion revealed approximately half the transgene expression when compared to homozygous plants, indicating that each allele contributes equally to total expression.
Additionally, lines were established which harboured the three different reporter genes on one T-DNA. Each of the genes was placed under the control of the CaMV 35S promoter. These transformants displayed comparable transcript levels for all three transgenes.

11-37 Transcriptional gene silencing of the tobacco LTR-retrotransposon Tnt1 in *Arabidopsis thaliana* as a model for the epigenetic control of retrotransposons in plants

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LTR-retrotransposons often constitute a large proportion of the repeated sequences of plant genomes. However, in the small *Arabidopsis thaliana* genome, class I transposable elements are less amplified and are localised preferentially in transcriptionally inactive heterochromatic regions (1), suggesting that the control of the amplification of these elements is particularly efficient in this species. To study the mechanisms preventing or limiting the amplification of LTR-retrotransposons in the plant genomes, we introduced the tobacco Tnt1 LTR-retrotransposon into Arabidopsis, thus mimicking the horizontal transmission of a new element in this model species genome. Tnt1 transposition events were induced at a high frequency during the transformation/regeneration process (2). Interestingly, when present at a high copy number in the Arabidopsis genome, Tnt1 is inactivated via a Transcriptional Gene Silencing (TGS) mechanism. This inactivation is associated with a high level of methylation of the transcribed sequence of the element.
Trans-silencing by TGS of a Tnt1-GUS translational fusion in F1 hybrids containing at least 3 copies of Tnt1 at the hemizygous state indicates that Tnt1 inactivation is dependent on the number of copies of the element. Tnt1-GUS inactivation is overcome by wounding, suggesting a direct effect of environmental stresses on the epigenetic control of retrotransposons in Arabidopsis.
We propose a model for the epigenetic control of retrotransposons in plants, in which transcriptional silencing would be mediated by double-stranded RNAs produced from aberrant RNA species, and leading to RNA dependent DNA methylation of homologous sequences.

(1) 2000 Nature, 408 (6814), 796 - 815.
(2) 2001 Mol. Genet. Genomics, 265, 32-42.

11-38 Transgene silencing in *Arabidopsis thaliana*: Triggers and mechanisms

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Predictable and stable transgene expression is a prerequisite for the broad use of transgenic plants. But in populations of transgenic plants variability of transgene expression over several orders of magnitude and even gene silencing is frequently observed.
For a comprehensive study of transgene expression in *Arabidopsis*, we established T-DNA lines harbouring different copy numbers of ß-glucuronidase or streptomycin-phosphotransferase genes under the control of the CaMV 35S promoter.
Below a certain number of identical transgenes we observed a positive correlation between copy number and reporter gene expression. However, silencing is triggered as soon as a certain copy number of a particular transgene is present in a transformant. Three copies of the GUS transgene are sufficient for the onset of silencing, whereas nine copies of the SPT gene are needed. Promoterless copies of a transgene do not induce silencing. These data indicate that the transcript level of a transgene has to surpass a probably gene-specific threshold for triggering of silencing.Hallmarks of post-transcriptional gene silencing were observed in silenced lines: small interfering RNAs, methylation of the transcribed transgene region and meiotic reversibility. Methylation of the transgene coding region was partially maintained despite meiotic reversibility of transgene silencing. In plants displaying silencing of the SPT gene truncated SPT transcripts were detected.

11-39 LIKE HETEROCHROMATIN PROTEIN 1: A new chromatin component in Arabidopsis

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Regulated gene transcription is central to developmental processes. This level of control occurs in part by the interaction of transcription factors with specific regulatory DNA sequences, but furthermore through gene silencing by heterochromatin formation.
LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) from *Arabidopsis* was recently isolated and characterised. This plant protein is a member of the HETEROCHROMATIN PROTEIN 1 (HP1) family as it has the chromatin organisation modifier (chromo) domain and the chromo shadow domain essential for the proper function of HP1 proteins.
Mutations at the *LHP1* locus affect general plant architecture, flowering time and leaf development suggesting that LHP1 may act as a regulator of gene expression during plant development and phase transition through formation of heterochromatin-like repressive complexes. This is suggested through the finding that a GFP-LHP1 fusion protein is distributed in foci throughout the nucleus similar to the punctuate localisation patterns observed for mammalian HP1. Secondly, that LHP1 is able to dimerise, an interaction shown to occur through the chromo shadow domain. Self-association of HP1 proteins permits heterochromatin formation and expansion leading to gene silencing in other eukaryotes and the situation may be similar in plants.

11-40 Drastic alteration of heterochromatin organization in interphase nuclei resulting from combination of gene silencing mutations

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Spatial distribution of interphase chromosomes contributes to the regulation of transcription and to the maintenance of epigenetic states. We examined the effects of mutations interfering with maintenance of transcriptional gene silencing (TGS) on heterochromatin organization in interphase nuclei using fluorescence *in situ* hybridization (FISH). Our probes specifically labeled centromeric or pericentromeric repetitive DNA or a transgenic locus affected by TGS. In wild type plants, all hybridization targets form constitutive heterochromatin that is assembled in DAPI-bright chromocenters, including the transgenic insert that forms a new heterochromatic knob. Such chromatin organization remains unaltered in the *mom1* mutant releasing TGS without significant changes of DNA methylation. In contrast, plants with decreased levels of methylation, caused by mutation of the *DDM1* gene encoding a SWI2/SNF2 related protein, exhibit significant decondensation of the centromeric and pericentromeric heterochromatin. Surprisingly, although *mom1* on its own has a nuclear organization indistinguishable from the wild type, nuclei of *mom1/ddm1* double mutants exhibit structural peculiarities not observed in the single mutants. Not only do the chromocenters disintegrate, but they also have the tendency to aggregate to a super-heterochromatic structure characteristic for the double mutant. The frequency of such heterochromatin collapse seems to be correlated with the severity of developmental abnormalities in the *mom1/ddm1* double mutant. This observation implies that MOM1 and DDM1 may have complementary functions in the maintenance of nuclear organization required for epigenetic inheritance and normal development.

11-41 An essential role of MSI1 in development

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MSI- and RbAp46/48-like proteins are components of several complexes involved in nucleosome assembly, histone acetylation or deacetylation and other chromatin modifying processes. In the model plant Arabidopsis, there are five genes encoding MSI-like proteins. Recently, one member of this family, MSI1, has been shown to form a complex with chromatin assembly complex (CAC) subunits CAC1 and CAC2 of Arabidopsis. The complex possesses nucleosome assembly activity in vitro. To elucidate the role of MSI1 in vivo, we constructed overexpressor and antisense lines. While plants with strongly elevated protein levels appear mostly normal, plants with reduced MSI protein levels show a diverse set of developmental alterations. The phenotype becomes progressively more severe through the plants life cycle, indicating a role of MSI1 in maintaining correct patterns of gene expression through successive rounds of cell divisions. Interestingly, the observed phenotypes only partially overlap with the phenotypes of the CAC1 and CAC2 mutants fas1 and fas2, strongly suggesting major roles of MSI1 in addition to chromatin assembly. In order to characterize the consequences of MSI1-deficiency or overexpression on global patterns of gene expression, transcriptional profiling experiments were performed using Affymetrix GeneChip® technology. Details of altered gene expression and the resulting developmental defects will be presented.

11-42 The meiotic defect of *Arabidopsis thaliana rad50* mutant

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The Rad50 protein (Rad50p) is involved in many mecanisms related with the maintenance of the genome stability : recombination, double-strand break repair, telomeres maintenance, cell cycle control and meiosis. In vertebrates Rad50p is essential for cell viability.
Our group has cloned the cDNA coding for the *Arabidopsis thaliana* Rad50p homolog and identified a T-DNA insertion *rad50* mutant. Using this mutant, we were able to demonstrate that most the functions of the Rad50p are conserved from yeast to plants, while the cellular lethality of the *rad50* mutation seems to be a specific feature of vertebrates.
In yeast, Rad50p null mutants have a meiotic defect which has a dramatic effect on spores viability and impaired synaptonemal complex assemblage. In *A. thaliana*, Rad50p mutation leads to both male and female sterility. Microscopic dissection of male meiosis in the anthers show a defect in early prophase I, as we were never able to observe later stages with fully condensed chromosomes and homologs pairing. This defect finally leads to chromosome fragmentation and the generation of polyads.
Fluorescence and differential contrast microscopy observations of the meiotic phenotypes of the *A. thaliana rad50* mutant will be presented.

11-43 AFLP based analysis of methylation profiles along Arabidopsis development

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In *Arabidopsis*, methylation has been related to silencing of gene expression and epigenetic gene inactivation. In fact, blocking DNA methylation through an antisense *METI* construct or by mutations at the *DDM1* locus results in a range of developmental abnormalities, including alterations in the flowering time of the plants. We have used AFLP analysis to follow the methylation state of anonymous CCGG restriction sites along plant development and as a result of different treatments. Sequence characterisation of a sample of the amplified fragments as well as the effect of demethylating agents or mutations at *DDM1* locus on the AFLP profiles, indicate that most detected fragments correspond to unmethylated CCGG sites. Methylation profiles are highly conserved among plants belonging to the same ecotype and vary in up to 34 % of the commonly detected sites between plants belonging to different ecotypes. The analysis of methylation profiles in different organs of the plant reveals that cotyledons are characterized by a high number of unmethylated sequences which become progressively methylated in consecutively produced rosette and inflorescence leaves. This AFLP based methylation profile analysis is also being used to characterize the effects of vernalization on cytosine methylation.

11-44 Characterization of a delayed floral organ abscission mutant

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Historically, delayed abscission was one of the first agricultural traits selected for by man. Abscission can be defined as the loss of an organ from the main body of the plant including the shedding of organs such as leaves, floral organs and fruits. Abscission is an active process during plant development in which cells separate from one another. Our model of abscission outlines four basic steps of development (ontogeny of abscission zone, competence to respond, activation of cell separation, and post abscission transdifferentiation)1. *dab4-1* (delayed abscission 4-1) was selected for delayed floral organ abscission by screening the Wisconsin T-DNA collection. *dab4-1* displays several unique phenotypes: it retains flowers until position 20, it is functionally male sterile due to a failure of pollen dehiscence and it displays strong apical dominance and delayed meristem arrest. *dab4-1* continues growing almost two times longer than wild type and more than 2.5 times taller. Almost 4 times more flowers are produced on the primary inflorescence. Genetic analysis indicates that a single recessive gene is responsible for the phenotype. The phenotype co-segregates with kanamycin resistance indicating the mutant is tagged or closely linked to the T-DNA insertion. We will present the phenotypic and physiological characterization of this mutant and preliminary genetic and molecular analysis.

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12-01 Factors regulating storage reserve synthesis and breakdown in Arabidopsis oilseeds

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The metabolic processes responsible for the accumulation and breakdown of storage lipid and protein reserves in arabidopsis seeds are under strict temporal and spatial control. We have isolated a number of mutants that are disrupted in these processes. A knock-out in trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for arabidopsis embryo maturation and is required for the expression of genes associated with seed storage reserve deposition (Eastmond et al., 2002, Plant J 29, 225-235). Characterisation of *tps1* has established for the first time that an enzyme of trehalose metabolism is essential in plants and is implicated in the regulation of sugar metabolism and embryo development. Storage lipids are converted to soluble sugars during germination through the action of several bioochemical pathways and organelles and we have isolated mutants from each of these. A block in peroxisomal fatty acid beta-oxidation completely blocks triacylglycerol breakdown from oil bodies whereas disruption of the glyoxylate cycle or gluconeogenesis does not block lipid breakdown and only compromises seedling establishment under sub-optimal growth conditions (Eastmond and Graham 2001, Trends Plant Sci., 6, 72-77). As regards regulation of lipid mobilisation to sugars we have recently shown that this continues even in the presence of ABA when seed germination is blocked (Pritchard et al., 2002, Plant J. In Press). In contrast, conditions of high carbohydrate and low nitrogen in the growth media can almost completely block lipid breakdown in seedlings that have germinated (Martin et al., 2002, Plant Physiology 128, 472-481). Metabolic regulation therefore plays a key role in the control of storage reserve synthesis and breakdown in arabidopsis seeds.

12-02 Biosynthesis genes of trehalose, a signalling molecule in Arabidopsis

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Trehalose is a non-reducing disaccharide that is very common in bacteria and fungi, but rarely found in plants. Only small amounts could be detected in Arabidopsis thaliana. In addition to its role as storage carbohydrate, trehalose levels have been shown to correlate very well with cellular stress resistance in many cell types and conditions. In the yeast Saccharomyces cerevisiae, trehalose is synthesised in two reactions from UDPglucose and glucose-6-phosphate by trehalose-6-phosphate (Tre6P) synthase (TPS1) and Tre6P phosphatase (TPS2). Deletion of tps1 eliminates growth on glucose because both TPS1 and Tre6P are indispensable for the regulation of glucose influx into the glycolysis. The two activities reside in a large complex together with a regulatory subunit redundantly encoded by the TSL1 and TPS3 genes. Following the publication of the complete A. thaliana genome sequence we have analysed the TIGRE database with multiple BLAST searches and found eleven TPS1 homologues. Interestingly, they can be grouped in two subfamilies, displaying most similarity either to yeast TPS1 or TPS2. No closely related homologues to the yeast TSL1 – TPS3 gene pair have been uncovered. Overexpressing AtTPS1 complements the yeast tps1? strain for its growth defect on glucose, which implies an important regulatory role for AtTPS1 in plant carbon metabolism. This functional complementation indicates also that AtTPS1 can replace yeast Tps1 in the trehalose synthase complex. We are currently investigating complex formation and the role of trehalose as a signalling molecule in Arabidopsis.

12-03 Knockout of the *TPT* gene of *Arabidopsis thaliana*: effects on primary metabolism and carbohydrate composition

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The *tpt-1* mutant was isolated by a reverse genetics approach and contains a T-DNA insertion 24bp 5 prime of the start ATG of the *TPT* gene, which codes for the chloroplast envelope triose-phosphate/phosphate translocator. The level of TPT mRNA, as well as of triose phosphate transport activity, is markedly decreased in the mutant. In *tpt-1* chloroplasts starch accumulates to higher concentrations than in the wild-type during the day, whereas the content of soluble sugars is decreased. In addition, a higher content of phosphorylated intermediates, but not of cytosolic Fru2,6BP, was observed. Measurements of 14C incorporation into starch indicate that starch turnover is increased in the mutant. Photosynthetic electron flow, quenching parameters, and CO2 assimilation were not altered under ambient CO2 conditions. However, when mutant plants were treated with high CO2 doses, their CO2 assimilation was reduced with respect to the wild-type. When the *tpt-1* mutation was introduced in the genetic backgrounds of *sex1-1*, affected in starch breakdown, or *adg1-1* which is impaired in starch biosynthesis, only in *tpt-1/adg1-1* a drastic drop in growth rate was observed. In the double mutant *tpt-1/sex1-1*, a higher content of high molecular weight polysaccharides was monitored. No changes in the level of mRNAs of other phosphate translocators were detected in the five genotypes. Taken together, our results indicate that in A. thaliana triose phosphate export from chloroplasts is not essential for plant primary metabolism, and that its lack can be compensated by other metabolic pathways such as an increased starch turnover.

12-04 Sugar response mutants with altered expression of the starch biosynthetic gene, *ApL3*

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Sugars are the metabolic link between carbohydrate production, utilisation and storage. They also co-ordinate these processes by regulating gene expression. For instance, high sugar levels suppress the expression of photosynthetic genes while inducing starch biosynthetic genes. How plants determine their sugar status is termed “sugar-sensing”; The components of the sugar signalling pathway are largely unknown, but interaction has been shown with the ABA and ethylene hormone signalling pathways. ADP glucose pyrophosphorylase is a starch biosynthetic enzyme with significant control over starch production. The expression of the *ApL3* gene, encoding a large subunit of the enzyme, is induced by sugar and modulated by ABA. We have identified mutants with altered sugar-regulated expression of this gene. The *ApL3* promoter fused to the coding region of a negative selection marker was used to isolate *impaired sucrose induction (isi)* mutants. Both ABA-related and novel sugar-responsive mutants have been identified in this screen. Conversely, mutants with enhanced sugar responses were selected using an *ApL3* promoter:: luciferase transgenic line. These *high sugar response (hsr)* mutants show luciferase and *ApL3* expression under low sugar conditions. This increased *ApL3* expression is still dose responsive to sugar and is not a result of non-specific up-regulation. These *hsr* mutants, like some of the *isi* mutants, appear to be unique to sugar responses and unconnected to hormone signalling pathways.

12-05 A plant P450 from genomics to function

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The 4 and 5-hydroxylations of phenolic compounds are catalyzed by cytochrome P450 enzymes. The 3-hydroxylation step, leading to the formation of caffeic acid from p-coumaric acid, remained however elusive, alternatively described as a phenol oxidase, a dioxygenase or a P450, with no decisive evidence for the involvement of either of them in planta. Analysis of genomic data, from phylogeny to EST frequency, pointed to CYP98A3 as the best possible candidate in the Arabidopsis genome. The CYP98A3 gene is highly expressed in inflorescence stems, roots and wounded tissues. Antibodies directed against recombinant CYP98A3 specifically reveal differentiating vascular tissues actively synthetizing lignin in stems and roots. The recombinant protein expressed in in yeast does not metabolize free p-coumaric acid, nor its glucose or CoA esters, p-coumaraldehyde, or p-coumaryl alcohol, which would be the likely substrates, but actively converts the 5-O-D-shikimate and quinate esters of trans-p-coumaric acid into the corresponding caffeic acid conjugates. The shikimate ester is converted 4 times faster than the quinate derivative. Further analysis of the catalytic properties of recombinant CYP98A3 shows that it also metabolizes the tyramine conjugate of p-coumaric acid. Taken together, these data show that CYP98A3 catalyzes the synthesis of chlorogenic acid, an antioxidant and defense molecule, and very likely also the 3-hydroxylation of lignin monomers. This hydroxylation occurs on depsides, the function of which was so far not understood, revealing an additional and unexpected level of networking in lignin biosynthesis, and a coupling between primary and secondary metabolism likely to play an important role in homeostasis.

12-06 Nucleotide metabolism in higher plants

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Pyrimidine and purine nucleotides are central metabolites in all species. They are part of nucleic acids and equally important is their role as a source of energy to drive numerous biochemical reactions. Nucleotides can be synthesized from amino acids and other small molecules (*de novo* pathway). Alternatively preformed pyrimidine and purine bases and their ribonucleoside derivatives can be recycled (salvage pathway) [1]. In *E. coli* and mammals nucleotide metabolism has been well characterised. However, not much is known about the individual genes and enzymes involved in the nucleotide metabolism of plants. Recent *in silico* analysis of *A. thaliana* and Southern analysis of *N. tabacum* and *S. tuberosum* revealed that most enzymes involved in the nucleotide de novo synthesis are encoded by single genes. In contrast, enzyme activities responsible for nucleotide salvage and phosphate transfer between purines and pyrimidines are encoded by gene families (Giermann et al., in press). It was suggested that changes in the contribution of nucleotide *de novo* synthesis and nucleotide salvage are under physiological and developmental control [2, 3]. Furthermore there are indications that the individual nucleotide pools are tightly regulated [3, 4]. The nucleoside monophosphate kinase URA6 isolated from yeast was shown to be specificic for both UMP and CMP with ATP serving as the phosphate donor [5]. This enzyme could therefore potentially regulate pool size and the phosphorylation state of both purine and pyrimidine pools. Here we report the cloning, over-expression in *E. coli* and characterisation of two monophosphate kinase isoforms isolated from *A. thaliana*, which show high sequence homology to URA6.

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12-07 Functional analysis of the members of the sulfurtransferase/rhodanese multi protein family in Arabidopsis

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Sulfurtransferases/rhodaneses (STs) are a group of enzymes widely distributed in plants, animals, and bacteria that catalyse the transfer of a sulfur atom from suitable sulfur donors to nucleophilic sulfur acceptors. The best characterized ST is bovine rhodanese which catalyses in vitro the transfer of sulfane sulfur from thiosulfate to cyanide, leading to the formation of sulfite and thiocyanate. However, in vivo neither substrates nor sulfur acceptors could be clearly identified in any of the organisms investigated. From Arabidopsis two very similar STs were isolated and characterized so far; these STs consist of two domains (N- and C-terminal) of nearly identical size connected by a short linker sequence, similar to the structure of the bovine rhodanese. By using different strategies to mine the databases 18 ST-like proteins containing typical rhodanese domains or signatures could be identified in Arabidopsis. Despite the presence of ST/rhodanese activities in many living organisms, the physiological role of the members of this multi protein family (MPF) has not been established unambiguously. The aims of this project are the isolation and characterization of all members of the ST/rhodanese protein family in Arabidopsis, the identification of their in vivo substrates and acceptors, and the determination of the respective reaction mechanisms. Finally, the function in the plant organisms of each single member of this MPF is going to be elucidated.

12-08 Identification and characterization of the Arabidopsis *PHO1* gene involved in phosphate loading to the xylem

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The Arabidopsis mutant pho1 is deficient in the transfer of inorganic phosphate (Pi) from the root epidermal and cortical cells to the xylem. The PHO1 gene was identified by a map-based cloning strategy. The N-terminal half of PHO1 is mainly hydrophilic while the C-terminal half has 6 potential membrane-spanning domains. PHO1 shows no significant homology to characterized solute transporter, including the family of H+-Pi co-transporters identified in plants and fungi. PHO1 shows highest homology to the Rcm1 mammalian receptor for xenotropic murine leukemia retroviruses, as well as to the Saccharomyces cerevisiae Syg1 protein involved in the mating pheromome signal transduction pathway. PHO1 thus defines a novel class of proteins involved in ion transport in plants. The gene is predominantly expressed in the roots and is weakly up-regulated under Pi-stress. Studies with PHO1 promoter-GUS constructs reveal predominant expression of the PHO1 promoter in the stelar cells of the root and the lower part of the hypocotyl. There is also GUS staining of endodermal cells that are adjacent to the protoxylem vessels. The Arabidopsis genome contains 10 additional genes showing homology to PHO1 and sharing similar hydropathy profiles. We are presently analyzing the expression pattern of all PHO homologues by RT-PCR as well as by promoter-GUS fusions and are aiming at analyzing the function of these genes in ion homeostasis through the isolation and characterization of insertion mutants.

12-09 Transporters of mineral nutrients: The Arabidopsis *NRT2* family

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Improving plant efficiency in nitrogen uptake and use requires a better knowledge of plant nitrogen assimilation pathway and its regulation. In our laboratory, a quantitative genetic studies of nitrogen use efficiency (see abstract from Loudet, Chaillou and Daniel-Vedele) and a physiological and molecular studies of nitrate transport were performed on the plant model Arabidopsis.
Three main steps are involved in nitrate assimilation in higher plants: nitrate uptake by root cells and nitrate reduction followed by nitrite reduction in roots or leaves. Nitrate uptake processes involve several components, namely low (NRT1) and high-affinity (NRT2) transporters which are currently being characterised molecularly in different species. Beside the high number of putative structural components, nitrate uptake is the target of complex regulatory processes, which are not yet clearly identified.
In *Arabidopsis thaliana*, a small multigenic family, containing 7 members that are distributed on three chromosomes, encodes putative high-affinity nitrate transporters. A T-DNA knockout mutant affected in the AtNRT2.1 and the AtNRT2.2 genes was isolated from the Arabidopsis insertion lines generated in Versailles and we demonstrated that these NRT2 genes are indeed involved in the high-affinity nitrate transport process. We analysed the relationship that exists between the 7 members of the family, at the level of both the We analysed the relationship that exists between the 7 members of the family, at the level of both the nucleotide and aminoacid sequences. Finally, we investigated the possible role of each member by analysing the expression pattern of each gene using semi-quantitative or real-time RT-PCR. Depending on the plant organ and/or the external nitrate availability, specific patterns were observed for some of these genes.

12-10 Loss-of-function *pdc1* plants are less tolerant to anoxia

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Ethanolic fermentation is an ancient pathway and serves in plants as a major route for the production of energy under anaerobic conditions. However, our recent research has shown that ethanolic fermentation has diverse functions under aerobic conditions as well. We were able to show, that during tobacco pollen development, fermentation genes are strongly expressed and acetaldehyde and ethanol are produced in fully oxygenated cells. Moreover, it was observed, that the alcohol dehydrogenase gene (*Adh*) from Arabidopsis is induced by a number of environmental stresses other than low oxygen, including dehydration, cold and absisic acid (ABA). We are studying several parameters of fermentation under these stress conditions: pyruvate decarboxylase (*Pdc*) gene expression, acetaldehyde and ethanol production. Results obtained by quantitative real-time RT-PCR identified *Pdc1* to be the major gene in the four members *Pdc* gene family, and to be induced by anaerobisis and by different abiotic stresses. The fermentation product acetaldehyde is detectable under anaerobic conditions and in low amounts under abiotic stress conditions. Loss-of-function *pdc1* mutants do not accumulate transcripts anymore. Furthermore, none of the other three *Pdc* genes show an altered expression pattern. *pdc1* mutants are less tolerant to anaerobic conditions, even more than the *adh* mutant. These results indicate clearly the major role PDC1 is playing in the activation of the fermentation pathway. Analysis for susceptibility of *pdc1* plants to drought, cold and salinity is currently in progress.

12-11 Characterization of the Nucleobase-Ascorbate Transporters family in *Arabidopsis thaliana*

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Purines play a key role in nucleic acid and nucleotide metabolism of all cells. In addition, they are used as nitrogen sources in many microorganisms and plants e.g. for the biosynthesis of secondary compounds or of ureides in legumes. On the other hand, ascorbate represents one of the main antioxidants in plants. Synthesized in the cytosol and in mitochondria, it accumulates to millimolar concentrations in different cellular compartments. Nucleobases and ascorbate, albeit structurally unrelated, are transported by a family of highly homologous transport proteins, the Nucleobase-Ascorbate Transporters (NATs). The *Arabidopsis thaliana*. genome contains at least 12 genes encoding putative NAT proteins with yet unknown functions. Two of the NAT proteins, NAT15 and NAT20, obviously contain transit peptide sequences. Interestingly, these two members are highly related to mammalian ascorbate transporters. *In vitro*. processing of both NAT15 and NAT20 precursor proteins with stromal extract could be observed, clearly showing that both proteins are directed to the plastids. By RT-PCR assays some members of the NAT family show ubiquitous expression, while other members show differential patterns of expression. To localize gene expression within the tissues, promotor-GUS fusions for some members were constructed. The transport specificities and the physiological roles of the various members of the NAT family is being investigated by heterologous expression of the proteins in yeast cells and the ascomycete *Aspergillus nidulans*. We also identified knock-out lines with T-DNA insertion for several of the NAT genes and the generation of double knock-down mutants by RNA interference (RNAi) is also in progress.

12-12 Arabidopsis *A BOUT DE SOUFFLE*, which is homologous to mammalian Carnitine Acyl Carrier, is required for post-embryonic growth in the light

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The degradation of storage compounds just following germination is essential to plant development, providing energy and molecules necessary for the building of a photosynthetic apparatus, allowing autotrophic growth. We identified A Bout de Souffle (bou) a new Arabidopsis mutation. Mutant plants stop developing post-germination, degrade storage lipids but cannot proceed to autotrophic growth. Neither leaves nor roots develop in the mutant. However, externally added sugar or germination in the dark can bypass this developmental block and allows mutant plant to develop. The mutated gene was cloned using the transposon Dissociation (Ds) as a molecular tag. The gene coding sequence shows similarity to the mitochondrial carnitine carrier (CACs) or CAC-like proteins. It is the first of its kind to be described in plants. In animals and yeast, these transmembrane proteins are involved in the transport of lipid-derived molecules across mitochondrial membranes for energy and carbon supply. The data presented here suggest that BOU identifies a novel mitochondrial pathway that is necessary to seedling development in the light. The BOU pathway would be an alternative to the well-known glyoxylate pathway.

12-13 Characterisation of *AtPht2-1*, a phosphate uniporter localized in the chloroplast

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Organic phosphate (Pi) is an important macroelement for plants, where it plays crucial roles in controlling key enzymatic reactions and regulating metabolic pathways. Several root specific genes encoding high affinity Pi-transporter were previously isolated. Pi transport is however not restricted to roots and also occurs in many other plant tissues (i.e. phloem, storage and reproductive organs) and organelles (chloroplasts, vacuoles). *AtPht2-1* encodes a novel low affinity Pi-transporter classified as a member of the PHT2 gene family. Northern blot and RT-PCR analysis revealed that *AtPht2-1* is expressed in green tissues, while no transcript could be detected in roots. The PHT2-1 protein contains a transit peptide sequence at the N-terminus. To localize the protein within the cell, in vitro import experiments were performed. In addition, the *AtPht2-1* cDNA was fused to a GFP reporter gene. These experiments showed that the protein is directed to plastids. For the better understanding of the function we isolated two Arabidopsis thaliana lines harbouring T-DNA insertions in *Pht2-1*. We present here their characterisation at the molecular, physiological and biochemical levels.

12-14 Circadian control of granule-bound starch synthase I gene expression. Effects on enzyme activity during the day/night cycle

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Granule-bound starch synthase (GBSSI) is one of the most extensively studied enzymes of the starch synthesis pathway and its role in the synthesis of amylose has been well stated. However, few studies have been carried out to characterize the regulation of GBSSI gene. Regulation of starch synthesis genes is especially interesting in photosynthetic tissues, where starch is subjected to a periodical alternation of synthesis and degradation during the day/night cycle. In this report we show a circadian oscillation of GBSSI mRNA levels in leaves of Arabidopsis thaliana during the day/night cycle, and provide evidences that GBSSI expression is controlled by the transcription factors CCA1 and LHY. Overexpression of both CCA1 and LHY genes causes the elimination of GBSSI mRNA oscillation and a decrease on the expression level of this gene. On the contrary, when expression of CCA1 and LHY is reduced and their amplitude of oscillation has been lowered (gi-2 mutant, affected in *GIGANTEA* gene), GBSSI mRNA levels show lower amplitude of oscillation and higher levels of accumulation. Binding shift assays indicate that this control may be exerted trough a direct interaction of those regulatory proteins with the GBSSI promoter. Oscillation is not observed on the GBSSI protein levels, which remains constant along the cycle. However, GBSSI activity shows a clear oscillation with a period of 24 hours that is altered in transgenic plants overexpressing CCA1. Further experiments are being carried out to discern whether this oscillation reflects a post-translational modification of the enzyme or is a consequence of changes in substrate availability when enzyme is getting buried in the growing starch granule. This work was supported by the Spanish Ministry of Education (Grant PB98-1122) and by Junta de Junta de Andalucía (PAI, group CV!-281).

12-15 Auxin mediates a disaccharide signaling in *Arabidopsis thaliana*

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We used sucrose analogs to study a sucrose-specific signaling pathway in *Arabidopsis thaliana*. Addition of sucrose analogs during the post-germinative phase results in an altered phenotype of the seedlings, showing reduced hypocotyl and root growth. The effects of sucrose analogs on gene expression was evaluated using DNA microarrays. Mutants insensitive to the analogs used were identified. Overall the results suggest that sucrose analogs mimic the effects of sucrose as a signaling molecule, and that the long-term effects observed in seedlings treated with the sucrose analogs are due to auxin overproduction as a consequence of sucrose sensing.

12-16 Tracking stress-induced changes in Arabidopsis metabolite patterns

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Most of the plant signaling molecules and many of the physiological changes elicited in plants by environmental changes remain unknown. An unbiased biochemical approach, using state-of-the-art analytical techniques will help identifying components of plant stress responses and plant intercellular communication. With the aim of elucidating plant stress responses and finding novel signaling molecules we established an experimental system to monitor the complexity of metabolic changes occurring in Arabidopsis under adverse conditions. Based on mainly LC-ESI-Qq-TOF-MS we are profiling (secondary) metabolites. Local and systemic tissues as well as the intercellular washing fluid of hydroponically grown plants are analysed. A few hundred mass signals can be resolved and detected in one LC-ESI-Qq-TOF-MS run. Using toxic metal exposure (abiotic stress) and challenge with bacterial pathogens (biotic stress) as model systems, we are identifying stress-induced changes. These analytical tools will also be valuable for gene function analysis by allowing the biochemical phenotyping of, for instance, mutants and different ecotypes of Arabidopsis thaliana or different developmental stages. Here we introduce our metabolite profiling and present exemplary (secondary) metabolite data on responses to varying degrees of toxic metal exposure.

12-17 Seed development and carbon metabolism in *Arabidopsis thaliana*

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This work is part of a research program aiming at identifying and studying genes involved in seed maturation using T-DNA insertion lines of *Arabidopsis thaliana* ecotype wassilewskija (WS). We focused on the WS wild type seed development so as to obtain a complete reference description of the seed metabolism, gathering both cytological observations of embryo development and biochemical composition of the seed. Complete developmental process, which can be roughly divided into three stages, will be described with particular emphasis on carbon metabolism. During embryo morphogenesis, large amount of starch accumulated transitorily, but storage compounds were almost absent. During maturation, a rapid increase in seed dry weight was observed, while a regular loss of water occured. Starch declined whereas triglycerides (and proteins) were massively synthesized. The second half of this maturation stage was characterized by the onset of sucrose storage. During late-maturation, storage compounds synthesis ended, concomittant with raffinose and stachyose accumulation, while a severe desiccation period led to a stage of quiescence. This study provided us with an integrated overview of the *A. thaliana* seed development. This physiological and biochemical reference data set will enable us to analyse mutants impaired in seed carbohydrate metabolism, and then to get further insights into the complex seed maturation process.

12-18 Identification and characterization of novel genes required for seed flavonoid pigmentation

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Our purpose is to analyze seed coat flavonoid nature and content in *Arabidopsis* and to study the influence of these pigments on seed quality (dormancy and longevity). To this end, we have been studying *Arabidopsis* mutants specifically affected in seed coat pigmentation. To date, 20 loci involved in flavonoid metabolism have been identified in *Arabidopsis*, which were mostly named *TRANSPARENT TESTA* (*TT*). Screening of the Versailles T-DNA-mutagenized *Arabidopsis* collection led us to identify 22 independent insertional lines showing a modification of the seed coat pigmentation. T-DNA tagged alleles were identified for four *TT* loci, namely *TT2*, *TT8*, *TT15*, and a novel locus named *TT16*. Previous studies demonstrated that both TT2 (a R2R3 Myb domain protein) and TT8 (a bHLH domain protein) modulate the expression of two late flavonoid biosynthetic genes, namely *DIHYDROFLAVONOL-4-REDUCTASE* (*DFR*) and *BANYULS* (*BAN*), in *Arabidopsis* siliques, supporting a major role of the TT2 and TT8 proteins in the flavonoid regulatory network (Nesi et al. 2000; 2001). Here, we focus on TT16 characterization. *TT16* encodes a MADS domain protein and its expression is restricted to the seed. A *tt16* null mutation prevented the activation of the *BAN* promoter in the endothelium layer, with the exception of the chalazal-micropylar area. In addition, mutant phenotype and ectopic expression analyses suggest that TT16 is also involved in the specification of endothelial cells. This study reinforces the conclusion that mutant which look like typical flavonoid mutants may actually provide the inroad to genes required for the development of specific tissues.

12-19 Characterization of a *wrinkled seed* mutant in Arabidopsis

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Mature seeds of *Arabidopsis* store proteins and up to 40% triacylglycerol (TAG), coming from the conversion of sugars, but lack starch. Regulation of oil quantity and partioning of carbon between storage products remain yet largely unraveled. To better understand these metabolic processes, an analysis of wrinkled seed mutants was undertaken in our group. One EMS mutant, which was provided by Dr. Christoph Benning (Michigan State University), was found to be seed specific. This line was named *wrinkled-2* (*wri-2*). *Wri-2* seeds show a large reduction in seed TAG but little change in fatty acid composition, a reduction in total seed protein but no changes in protein classes, and a reduction in sugar content during embryo development. However, detailed analyses using confocal scanning microscopy revealed that primary effect caused by the *wri-2* mutation is in the development of the seed endosperm. Indeed, *wri-2* lacks chalazal endosperm and contains large and irregular nucleo-cytoplasmic domains, which do not migrate properly to the periphery of the central cell. In addition, mitosis in endosperm is altered and improper cellularization of the endosperm is observed. In conclusion, the low levels of storage products in *wri-2* seeds support the idea that proper endosperm development is essential for normal storage product accumulation in the embryo. Genetic analyses demonstrated that *wri-2* is a leaky single gene mutation with no parental or gametophytic effects. To gain further insights into WRI-2 nature and possible function(s), fine mapping of *wri-2* has been carried out and cloning of the corresponding gene is underway.

12-20 Isolation and characterization of cDNAs encoding the protein subunits of Arabidopsis RNase P

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RNase P is a ubiquitous enzyme involved in processing precursor tRNAs to form mature tRNAs. In bacteria, this endoribonuclease comprises one catalytic RNA moiety and one protein cofactor. In contrast, both yeast and human nuclear RNase P contain a single RNA subunit but nine and ten protein subunits, respectively. Although RNase P functions as a ribonucleoprotein complex in all living organisms, the need for increased complexity of the eukaryal enzyme remains unclear. Plants might serve as ideal model systems to investigate the spatial, temporal and developmental regulation of expression of various subunits and the individual contribution of each subunit to function of the RNase P holoenzyme. Therefore, we have begun an extensive investigation into the biochemical and genetic characterization of plant RNase P. Towards the latter objective, we have used the amino acid sequences of the protein subunits of yeast and human RNase P as query sequences in BLAST searches and identified the full-length or partial sequences of the putative homologs in different monocot and dicot plants. Based on the information retrieved from these searches, we designed appropriate oligonucleotide primers for either RT-PCR-based amplification of cDNAs or RACE-based techniques to unambiguously identify the 5´ and 3´ ends of the cDNAs of interest. Such approaches have facilitated identification of the sequences of the complete ORFs for at least five of the putative protein subunits of *Arabidopsis* RNase P. Comparison of the amino acid sequences of the protein subunits of plant RNase P with their corresponding archaeal and eukaryal counterparts have provided insights with regard to their function. Currently, we are employing a genetic strategy in yeast to examine if the *Arabidopsis* cDNAs we isolated can functionally complement their yeast counterparts.

12-21 Role of *BOR1* in xylem loading of B

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Arabidopsis thaliana bor1-1 mutant is sensitive to B deficiency and has a defect in xylem loading of B. *BOR1* was identified based on the map position and the cloning was further confirmed with complementation test. BOR1 is a membrane protein with ten putative transmembrane domains. There are six homologous genes present in the genome of *Arabidopsis thaliana*. Homologous genes are also present in rice, soybean, maize, etc., suggesting that the gene is common among plants. Expression of *BOR1* is detected in roots, strong expression was observed in pericycles. Expression of BOR1 in yeast resulted in reduction of B cocentration in cells. B is mostly taken up by plants as boric acid. Boric acid is a non-charged molecule at neutral pH and has relatively high membrane permiability. Taken together with our results, it was suggested that B is taken up by roots mostly by passive mechanism and reach near xylem where it was transported by BOR1 or related protein into xylem for transport into shoots.

12-22 *AtMRU1* and *AtMRD1*, two novel genes with altered mRNA levels in a methionine over-accumulating *Arabidopsis thaliana* mutant

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The *Arabidopsis thaliana mto1-1* mutant over-accumulates soluble methionine (Met) up to 40-fold higher than that in wild-type Col-0 plants. In an effort to identify genes regulated according to changes in methionine (Met) accumulation and determine the extent of Met influence on plant development, DNA microarray analysis of young rosettes and developing siliques of the *mto1-1* mutant was carried out. From this analysis, 45 genes were selected for further study. Expression of these genes was then examined in detail in three developmental stages of the *mto1-1* mutant using a combination of northern hybridisation and real-time PCR. Eight genes were identified with consistently altered mRNA levels in at least one developmental stage of the *mto1-1* mutant. This included two novel genes referred to as *AtMRU1* and *AtMRD1* that have no clear ORF or similarity to previously reported genes. *AtMRU1* is up-regulated three-fold in young *mto1-1* rosettes and exhibits a developmental response to the *mto1-1* mutation. In contrast, *AtMRD1* mRNA levels were strongly down-regulated in *mto1-1* and approximately 10-fold lower in rosette and seed tissues compared to that in wild-type. Preliminary data suggests that the down-regulation of *AtMRD1* involves a transcriptional control and that the -459 bp to +19 bp region of the *AtMRD1* gene promoter is sufficient for this activity. Current data on the analysis of *AtMRU1* and *AtMRD1* will be presented.

12-23 The cysteine synthase complex- a metabolic sensor for the regulation of sulfate assimilation

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Cysteine represents the exclusive precursor for reduced sulfur containing plant metabolites and secondary compounds, e.g. methionine, proteins, glutathione, phytochelatins or glucosinolates. The formation of cysteine is initiated by serine acetyltransferase (SAT) that generates the free intermediate O-acetylserine (OAS). OAS is subsequently used by O-acetylserine (thiol) lyase (OAS-TL) for the incorporation of sulfide into cysteine. SAT and OAS-TL together form the hetero-oligomeric cysteine synthase complex (CSC) but no substrate channeling of OAS between both enzymes is observed. The dissociation of the CSC by OAS results in drastic changes of enzymatic activities of both enzymes. Therefore the function of the complex might be to serve as a regulatory switch for the control of flux through the cysteine synthesis and even sulfate assimilation pathway. To characterize the dissociation in response to the putative effector OAS we have applied surface plasmon resonance based analysis with the BIAcore system and monitored real-time protein-protein interaction in the mitochondrial CSC of *Arabidopsis thaliana*. Determination of the association and dissociation rate constants yielded the equilibrium dissociation constant (KD = 25 x 10-9M) based on a reliable A + B <-> AB model. The effect of OAS on dissociation of CSC showed a strong positive cooperativity and a half maximal dissociation rate of about 80µM OAS that corresponds to physiological OAS concentrations. These findings provide a quantitative basis for the function of the CSC. It is discussed that the CSC acts a molecular sensor system that monitors the sulfur status of the cell and controls sulfate assimilation and cysteine synthesis according to the availability of sulfate.

12-24 Enzymes of the branched-chain amino acid metabolism in *Arabidopsis thaliana*

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Plants are capable of synthesizing all branched-chain amino acids. Catabolic pathways are less clear and only recent investigations have revealed the enzymes for degradation of leucine and possibly other branched-chain amino acids. Towards a better understanding of the enzymes involved in these pathways and their regulatory control in Arabidopsis, we presently investigate the branched-chain aminotransferases (AtBCAT) and the isovaleryl-CoA dehydrogenase (AtIVD). While the former are present in at least six isoforms, the AtIVD is encoded by a single gene. Studies with yeast complementation assays and substrate specificity tests with recombinant proteins showed the activities and contribution of these enzymes in plant branched-chain amino acid metabolism. Beside the spatial separation of the biosynthetic (in chloroplasts) and catabolic (in mitochondria) pathways within the cell, sucrose and other metabolites seem to have reverse effects on the expression of at least some of the enzymes of the counteracting branches in this metabolism. In vivo analysis of the AtIVD promoter with GUS fusion constructs revealed a tissue-specific repression of the promoter activity by sucrose in above ground parts of the plant. This suggests that the net direction of branched-chain amino acid metabolism is influenced by carbon sources.

12-25 Posttranscriptional autoregulation of cystathionine gamma-synthase, the committing enzyme for methionine biosynthesis: in vitro studies

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Studies with Arabidopsis *mto1* mutants that over-accumulate soluble Met revealed that the stability of Cystathionine gamma-Synthase (CGS) mRNA is downregulated in response to Met application and that the *mto1* mutation abolishes this regulation. All *mto1* mutants carried single-amino acid alterations within the first exon of CGS. Transfection experiments showed that the CGS exon 1 coding region is necessary and sufficient for this regulation and that it is the amino acid sequence that has a role in this regulation. Furthermore, genetic crosses indicated that CGS exon 1 acts in cis. A plausible model is that this regulation occurs during translation when the nascent polypeptide of CGS exon 1 and its mRNA are in close proximity. Deletion and Ala-scanning mutagenesis showed that the functional region for this regulation is 11-13 amino acids, or (A)RRNCSNIGVAQ(I), which is highly conserved among plant CGS but cannot be found elsewhere in the database. In order to explore the molecular mechanism of this regulation, we adopted wheat germ in vitro translation system and showed that the effector for this regulation is S-adenosylmethionine (SAM), a direct metabolite of Met. When wild-type CGS exon 1 was translated in the presence of SAM, northern analysis detected a 5'-truncated RNA that was similar to that observed in vivo (Chiba et al. Science 286: 1371-4, 1999). Hybrid-selection at the 5'-region detected a short RNA that seems to be the counter part of the 5'-truncated RNA. The results suggest that an endonuclease is involved in this regulation.

12-26 Sensitive and high throughput metabolite assays for inorganic pyrophosphate, ADPGlc, nucleotide phosphates, and glycolytic intermediates based on a novel enzymic cycling system

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Metabolite assays are required to characterise metabolic changes during development, after an environmental perturbation or in a particular genotype, and to identify sites for regulation and investigate the underlying mechanisms. The minute size of Arabidopsis seeds poses a technical challenge for such studies. A set of assays using a novel enyzmic cycling system based on glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate oxidase have been developed and optimised for use with growing Arabidopsis seeds. They are combined with other existing assays to provide a suite of high throughput, sensitive assays for the immediate precursors for starch (adenine diphosphate glucose) and lipid (acetyl coenzyme A and glycerol-3-phosphate) synthesis, as well as pyrophosphate, ATP, ADP and most of the glycolytic intermediates. A method is presented to allow rapid quenching of intact Arabidopsis siliques, followed by manual separation of the seeds from the remainder of the silique. These assays are used to investigate the changes in metabolite levels in seeds and pod walls during development and maturation, and in response to a stepwise decrease of the external oxygen concentration.

12-27 Molecular evolution of functionally improved lipoxygenases

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Plants metabolize polyunsaturated fatty acids into a range of compounds termed “oxylipins”. These compounds play important roles in plants´ responses to pathogen invasion, pest attack and wounding, and contribute to the key aroma and flavour compounds of fruits and vegetables. The first step in the oxylipin pathway is carried out by lipoxygenase (LOX; E.C. 1.13.11.12, linoleate: oxygen oxidoreductase). LOX is a non-haem iron-containing dioxygenase that catalyses the oxidation of polyunsaturated fatty acids to produce 9- or 13- fatty acid hydroperoxides. All plants contain multiple isoforms of LOXs. Six different genes encoding LOX have been identified in the Arabidopsis genome. The sequence diversity of the two published Arabidopsis LOXs suggests that they may play different roles within the plant. LOX exhibit positional and chiral specificity which are dependent on the source of the enzyme, and which determine the nature of the subsequent oxylipins formed from the hydroperoxides and, consequently, the biological significance of the LOX specificity. In order to better understand these issues and to obtain new enzymes with enhanced properties as biocatalysts we are currently using two well characterized LOXs from pea to develop a protocol for forced molecular evolution of LOX based on the DNA “Shuffling” technique. We intend that the shuffled sequences will help us to better understand the basis of substrate and product specificity and also provide new species with altered specificities that can be used either as new biocatalysts or to manipulate oxylipin metabolism in Arabidopsis.

12-28 Phosphoenolpyruvate carboxylase gene family from *Arabidopsis thaliana*: Analysis of gene expression and characterization of the encoded proteins

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Phosphoenolpyruvate carboxylase (PEPC) catalyses the beta-carboxylation of PEP in a reaction that yields Pi and oxaloacetate. PEPC plays an important role in C4 and CAM photosynthetic carbon metabolism. In C3 plants, this enzyme is found in most organs and, among other roles, it plays an anaplerotic function which consists of the replenishment of oxaloacetate in the tricarboxylic acid cycle whenever the demand of carbon skeletons for amino acid biosynthesis is high. A search of *Arabidopsis* genome identified three genes putatively encoding phosphoenolpyruvate carboxylase here stated *Atppc1* (in chromosome I), *Atppc2* (in chromosome II) and *Atppc3* (in chromosome III). These genes show a high degree of similarity (about 75 %) and encode polypeptides of 107 kDa (AtPEPC2) and 110 kDa (AtPEPC1 and AtPEPC3). Northern blot analysis using as probe the cDNA corresponding to exon 8 of *Atppc3* identified a single band, most probably corresponding to the accumulation of transcripts of the three genes, and revealed a highest accumulation of PEPC transcripts in the root. Relative RT-PCR analysis showed differential expression of PEPC genes in *Arabidopsis*: *Atppc1* and *Atppc2* are expressed in all plant organs, however, *Atppc3* showed specific expression in roots. The coding region of the three PEPC genes were expressed in *E. coli* as His-tagged proteins. The three recombinant polypeptides showed PEPC activity and were efficiently identified by a polyclonal antibody raised against the *Shorgum*C4 PEPC. We are now characterizing the kinetic parameters of each of the purified His-tagged PEPC polypeptides and their post-translational modifications.

12-29 Positional cloning of *FLU*, a gene of *Arabidopsis thaliana* that encodes a negative regulator of chlorophyll biosynthesis

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Tetrapyrroles such as chlorophylls play a fundamental role in the energy absorption and transduction activities of photosynthetic organisms. Because of these molecules, however, photosynthetic organisms are also prone to photooxidative damage. They had to evolve highly efficient strategies to control tetrapyrrole biosynthesis and to prevent the accumulation of free intermediates that potentially are extremely destructive when illuminated. In angiosperms, the metabolic flow of tetrapyrrole biosynthesis is regulated at the step of 5-aminolevulinic acid (ALA) formation (Granick, 1959). In the dark, the chlorophyll biosynthesis pathway leads only to the formation of protochlorophyllide, since NADPH:protochlorophyllide oxidoreductase, the enzyme that converts protochlorophyllide to chlorophyllide, requires light as a cofactor (Reinbothe et al., 1996). Once a critical level of protochlorophyllide has been reached, ALA synthesis slows down. Using a genetic approach we isolated an Arabidopsis thaliana mutant, flu, that is no longer able to suppress the accumulation of protochlorophyllide in the dark. The positional cloning strategy was used to isolate the FLU gene. The identification of FLU was confirmed: 1) single point mutations were found in this gene of four allelic flu mutants and 2) the flu phenotype was complemented by introducing the wild type copy of the gene into mutant plants. In the Arabidopsis genome, FLU is a single gene and not a member of a gene family. It encodes a novel, previously not described protein. The FLU protein is very likely located in plastids since it was imported and processed by isolated pea chloroplasts. FLU was tightly associated with chloroplast membranes. Two domains, coiled-coil and tetratricopeptide repeat (TPR) that are known to mediate protein-protein interactions, were predicted in FLU. In the flu1-1 and flu1-4 mutants, the amino acid exchanges were found in the TPR and coiled-coil domain, respectively. Therefore, these domains might be functional units. The predicted features of the FLU protein suggest that FLU might confer its regulatory effect through interaction with another protein(s), possibly with enzyme(s) of chlorophyll biosynthesis. This hypothesis is currently under investigation.

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12-30 A root-specific condensing enzyme from *Lesquerella fendleri* that elongates very-long-chain saturated fatty acids

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Very-long-chain fatty acids (VLCFAs; chain lengths >18) are synthesized by sequential additions of two carbon moieties from malonyl-CoA to C18 fatty acyl precursors. Each cycle of elongation involves four enzymatic reactions, the first of which is a condensation reaction. It is the substrate specificity of the condensing enzyme that determines the spectrum of VLCFA products made in a given cell type. A gene, *LfKCS45*, was isolated by probing a *Lesquerella fendleri* genomic library with the *Arabidopsis FAE1* gene. *FAE1* encodes a seed-specific condensing enzyme. At the amino acid level LfKCS45 was found to share 70% sequence identity with *Arabidopsis* FAE1. Fusion of the *LfKCS45* promoter to the *uidA* reporter gene resulted in expression of beta-glucuronidase activity exclusively in the root-tip region of transgenic *Arabidopsis* plants. Expression of the *LfKCS45* in *Saccharomyces cerevisiae* lead to an increase in the accumulation of saturated VLCFAs (>C26). *Arabidopsis* plants have been transformed with *LfKCS45* driven by a seed-specific promoter. The fatty acid profile of seeds from the transgenic lines is currently being analyzed. The substrate specificity of LfKCS45 is also being investigated using *in vitro* assays.

12-31 The Arabidopsis *MYB* gene *AtMYB32* may be involved in pollen development and anthocyanin biosynthesis

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The transcription factor gene AtMYB32 from Arabidopsis thaliana encodes a MYB family protein with the conserved N-terminal R2R3 DNA-binding (MYB) domain. AtMYB32 has homology with several MYB genes that have been implicated in phenylpropanoid biosynthesis. Analysis using RT-PCR shows AtMYB32 is expressed in flowers, leaves, stems and roots. Promoter-reporter gene studies on AtMYB32 using GUS have shown expression to be strongest in the anthers, pollen and style of flowers, developing lateral roots and young leaves of seedlings. Expression in mature leaves is evident in the mesophyll cells and strongest at regions of excision (wounding). Using RNA interference and T-DNA insertion an aborted pollen phenotype has been produced. Plants carrying the transgene and plants with an insertion in the 3´ untranslated region produce up to 50 and 100% aborted pollen respectively. The pollen are non-viable and siliques of plants carrying the insertion are truncated. Studies on promoter-GUS lines using plant hormones have shown that A tMYB32 expression is strongly enhanced in roots by auxin (and analogues) after 24 hours. Enhancement occurs only in the presence of sucrose. RT-PCR and microarray analysis on mutants indicate involvement in anthocyanin biosynthesis and the pathogenic response, and reveal several potential target genes.

12-32 Sensing of sulfur nutrition in higher plants: Profiling of Arabidopsis Sultr1;2 sulfate transporter knockout mutant

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Sulfate uptake in roots is the initial and crucial step of sulfur assimilation in plants. During sulfate starvation, transporters and assimilatory enzymes are up-regulated both at the mRNA and protein levels. In *Arabidopsis*, high-affinity sulfate transporters, Sultr1;1 and Sultr1;2 that exist in the root epidermis and cortex are involved in the initial uptake of sulfate from the soil, and their mRNA levels are highly regulated responding to the external sulfur conditions. Inhibitor studies suggested that increase of Sultr1;1 mRNA is suppressed by inhibition of transcription and protein phosphatase activities upon sulfur starvation. Sultr1;1 mRNA was abundantly accumulated in the T-DNA insertion mutant of Sultr1;2, suggesting that internal sulfur status may control the level of Sultr1;1 mRNA. Sensing of sulfate levels and regulatory mechanisms of gene expression responding to sulfur availability have not been well characterized in higher plants. Transcriptomic profiling of sulfur-responsive genes is carried out through DNA chip analysis of the Sultr1;2 knockout mutant.

12-33 Detection of intra- and intermolecular interactions between sucrose transporters

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Sucrose represents the major transport form for carbohydrates in plants. Sucrose is transported in sieve elements, which constitute the conduit for assimilate export out of leaves. Three members of the sucrose transporter family (SUTs) with different kinetics were identified: SUT1, a high affinity sucrose proton co-transporter, SUT4 a low affinity transporter and SUT2, which in yeast is only weakly active and show features reminiscent of yeast sugar sensors RGT2 and SNF3. Immunolocalization on serial sections demonstrates that all three SUT proteins are localized in the same enucleate sieve element. Thus the potential of sucrose transporters to form homo-oligomers was tested by the yeast-based split ubiquitin system. Both SUT1 and SUT2 have the potential to form homo-oligomers. Moreover, all three sucrose transporters have potential to interact with each other. Interactions seem to be specific since a plasma membrane potassium channel and a glucose transporter did not interact with SUTs. Intramolecular interaction of SUTs was further analyzed by separate expression of sucrose transporter halves using the split ubiquitin system and functional assays. Separately expressed halves reconstitute sucrose transport activity at the plasma membrane with affinities similar to the intact protein. Furthermore, the N-terminal half of the low affinity SUT2 interacts functionally with the C-terminal half of SUT1, resulting in a protein with low affinity for sucrose. The in vivo interaction between the functionally different sucrose transporters indicates that the membrane proteins are capable of forming hetero-oligomeric structures that, similar to mammalian glucose transporter complexes, might be of functional significance for transporter regulation.

12-34 AtNRT1:4, a petiole nitrate transporter, is important in regulating nitrate distribution

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CHL1 is a dual-affinity nitrate transporter gene isolated by a T-DNA tagged nitrate uptake mutant. *AtNRT1:4* is one of 52 CHL1 homologs found in Arabidopsis genome which shares 53% amino acid sequence identity to *CHL1*. Electrophysiological studies of *AtNRT1:4* cRNA-injected Xenopus oocytes indicate that AtNRT1:4 is a pure low affinity nitrate transporter. Whole mount in situ hybridization and northern blot analyses demonstrate that the expression of *AtNRT1:4* is located in cortical cells of leaf petiole, and is not induced by nitrate. A T-DNA inserted *atnrt1:4* mutant exhibits wrinkle leaf surface and yellow-green leaf edge. Nitrate uptake level of *atnrt1:4* mutant is the same as that of wild type when plants are grown in (NH_4) 2Succinate containing medium. However when plants are grown in NH_4NO_3 containing medium, nitrate uptake level of *atnrt1:4* mutant is lesser than that of wild type. Since *Atnrt1:4* is not expressed in the roots, reduced nitrate uptake activities of mutant grown in NH_4NO_3 might be caused by feedback regulation. Nitrate content ratio of root/shoot is higher in *atnrt1:4* mutant than in wild type. In contrast with higher nitrate content in petiole and nitrate content gradually decreasing toward the front edge of wild type leaves, nitrate distribution in the leaves of *atnrt1:4* mutant is the other way around. Taken together, our data indicated that 1) Leaf petiole nitrate transporter AtNRT1:4 is important in retrieving nitrate from xylem, and 2) Petiole is important in regulating nitrate distribution.

12-35 Characterisation of Arabidopsis acetate non-utilising mutants uncovered an AMP-binding protein with acyl-CoA synthetase activity

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A germination based screen of a T-DNA mutated population of *Arabidopsis thaliana* was used to isolate plants resistant to monofluoroacetic acid, a toxic analogue of acetate. The mutations of two independently isolated lines were shown to be both recessive and allelic. Identification of the site of insertion by TAIL PCR revealed that the phenotype was due to disruption of a putative peroxisomal AMP-binding protein located on chromosome III. The putative AMP-binding protein was cloned from a cDNA library and expressed in *E. coli*. The 60kDa protein product was demonstrated to have short-chain acyl-CoA synthetase activity. Gene expression analysis using the mutant provided further evidence that disrupting organic acid utilisation perturbs the perception of carbohydrates in newly germinated seedlings.

12-36 Carbohydrate perception is altered in acetate non-utilizing mutants

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The toxin monofluoroacetic acid was employed to isolate Arabidopsis plants deficient in their ability to utilize or sense acetate. Following conventions established from related fungal work, the mutants were called *acn* mutants for *ac*etate *n* on-utilization. Three highly resistant lines were the focus of genetic and physiological studies. Mutant *acn1* appears to be a true acetate non-utiliser as it displays increased sensitivity to exogenous sodium acetate. Mutants *acn2* and *acn3* appear to be sensitive to exogenous sucrose. Seeds of *acn2* showed germination behavior that was sucrose concentration dependent. Seeds did not germinate without exogenous sucrose or with 100 mM sucrose, but 50-60% of seeds germinated and established normally with 20 mM sucrose. Young *acn3* seedlings showed significant chlorosis at 20 mM sucrose, but no chlorosis in the absence of exogenous sucrose. This phenotype was alleviated if acetate was provided. The *acn* mutants demonstrate that disrupting organic acid utilization has profound affects on carbohydrate metabolism.

12-37 Plant polysaccharide biosynthesis: Further insights into the pectic component rhamnogalacturonan

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Pectins are complex and partly branched polysaccharides found in the primary cell walls of all higher plants. Major pectic components are homogalacturonan and the rhamnogalacturonans RGI and RGII. The former is made up entirely of galacturonic acid units, whereas the latter two also contain considerable amounts of rhamnose units. Even though the structural composition of these components has been known for quite some time, their biosynthesis remains largely obscure. So far it is only postulated that these compounds are built from NDP-sugar precursors by the action of sugar-transferases located in the Golgi-apparatus. But the enzymatic machinery for the synthesis of these NDP-sugars remains largely uncharacterized. Known bacterial genes involved in the synthesis of dTDP-rhamnose were studied in an attempt to identify genes involved in the biosynthetic pathway of the analogous plant glycoconjugate UDP-rhamnose. One of these genes is rmlB, which harbors close homology to three Arabidopsis genes, termed RHM1 to RHM3 [1]. Due to the sequence similarity the RHM products are postulated to play an important role in the commitive step of the conversion of UDP-glucose to UDP-rhamnose. Insights into the function of these genes are obtained by determining the expression level and location of expression of one of these genes, altering its expression level in transgenic plant experiments, followed by characterization of the concurrent effects on the pectic components of the plant cell wall.

[1] Reiter, W.D. and Vanzin, G.F. Plant Mol Biol 2001 Sep; 47(1-2): 95-113

12-38 The Arabidopsis V-ATPase and its role in cell expansion

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The V-ATPase is a highly conserved eukaryotic proton pump, whose primary function is the acidification of endomembrane compartments. It is essential for many processes in the cell including ion homeostatis, secondary active transport, enzyme activity, protein targeting, and vesicle trafficking. Using hypocotyls and pollen tubes as model systems, we are focusing on the role of the V-ATPase in cell expansion. We have used the V-ATPase-deficient mutant *det3*, as this mutant has a de-etiolated phenotype and a dwarf stature, which can largely be ascribed to defects in cell expansion. However, when grown in the dark under certain conditions,the mutant shows a normal etiolated phenotype, indicating that the phenotype is conditional. Using Concanamycin A, a specific inhibitor of the V-ATPase, the short hypocotyl phenotype can be mimicked in wt seedlings. This makes it possible to study the effect on living cells immediately after inhibition of the V-ATPase, using microscopy and biochemical methods. We are also investigating the role of subunit C for enzyme activity and stability under various conditions using immunoblotting and activity measurements. In addition, we have identified concanamycin-insensitive mutants, the study of which hopefully will bring new insights into the regulation of the V-ATPase.

12-39 Pectin methylation and leaf morphology in wild-type and Adenosine kinase-deficient plants

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We are studying the pattern of pectin methylesterification during leaf development in Arabidopsis wild type and adenosine kinase-deficient (ADK) plants and its association with leaf morphology. The carboxyl groups of homogalacturonan (HG) within pectin are highly esterified when first synthesized to facilitate cell expansion; later, pectin esterification decreases due to the action of pectin methylesterases in the cell wall, and calcium cross-bridges form between HG groups. Our earlier results show that reduced ADK activity results in an inhibition of transmethylation activity in Arabidopsis. For example, the pectin within the seed coat mucilage of these plants has a lower degree of methylesterification than that of wild-type seed. The leaves of the ADK-deficient lines have a very distinct morphology: they are smaller and quite wrinkled. We are investigating now whether this phenotype due to reduced pectin methylesterification that limits cell expansion irregularly. Immunocytochemistry using JIM5 and JIM7, monoclonal antibodies to relatively low and high methylesterification states of HG respectively, is being used in an attempt to explain the altered leaf morphology of the ADK-deficient lines. A direct GC-MS analysis of HG esterification in young and old leaves of wild type and ADK-silenced lines is also being carried out. These studies will complement a morpho-anatomical and ultrastrutural comparison of the two genotypes to evaluate the timing and degree of pectin methylesterification with respect to their leaf morphologies.

12-40 Transcript co-response analysis of *Arabidopsis thaliana* tricarboxylic acid cycle genes

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Many technologies for multi-parallel gene expression analysis are nowadays in use and data sets from diverse experiments made available via the internet. These data provide a rich resource for cross-experiment investigation of correlated behaviour of steady-state mRNA levels of genes of interest under a wide spectrum of experimental conditions. While analysis of gene homology is a routine procedure used for gene annotation, functional prediction can now be extended towards sets of non-homologous genes based on the assumption that co-ordinately regulated gene expression is indicative of common function.
In this work we initially focused on genes from *Arabidopsis thaliana* which were known to belong to the tricarboxylic acid (TCA) cycle pathway. We attempted to identify so-far unknown candidate genes which appeared to be associated with the TCA cycle and might have regulatory function.
For this strategy we used combined publicly available and in-house gene expression data from different gene expression technology platforms. Pearson´s linear correlation coefficients, as well as Spearman and Kendall rank-order correlation coefficients were computed from a complete dataset of 340 different experiments. Significance thresholds based on Bonferoni- and Dunn-Sidak-type corrected alpha errors for multiple tests were used to accept or reject gene correlations. Euclidian distance was used as additional independent distance measure commonly used for definition of gene co-response behaviour. Furthermore, analysis was performed on subsets of experiments representing common ecotypes. Significant correlations were used to generate co-response behaviour. Furthermore, analysis was performed on subsets of experiments representing common ecotypes. Significant correlations were used to generate co-response networks which were visualized and processed with the Pajek software for analysis of large networks.
In this poster presentation we show first results of our analysis of plant TCA cycle genes and evaluate identified gene co-responses by statistical methods.

12-41 Sphingolipid signalling in plants: Identification of the delta-4 sphingoid base desaturase

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The T1J1.1 gene was identified from the Arabidopsis sequencing project on the basis of its homology to many fatty acid desaturase enzymes. There are a number of orthologues of T1J1.1 throughout higher eukaryotes, but studies to determine the function of some of these have proved inconclusive. We have been studying the Arabidopsis gene and its *Schizosaccharomyces pombe* orthologue. Expression of the *S. pombe* gene in a yeast expression system revealed it to be a delta-4 sphingolipid long chain base (LCB) desaturase gene. The delta-4 LCB desaturase makes sphingosine, the precursor to sphingosine-1-phosphate (S-1-P)- a highly potent signalling molecule in many biological systems. S-1-P has been implicated as a signalling molecule in a number of responses in animals including cell growth and proliferation and apoptosis, and in stress responses and apoptosis in higher plants. In order to study the plant orthologue, transgenic Arabidopsis plants have been produced with the T1J1.1 cDNA overexpressed in both sense and antisense orientations. Homozygous antisense lines display a marked dwarf phenotype compared to wild-type plants. This poster will detail phenotypic analysis of the antisense lines, and the endogenous expression of the *S. pombe* gene under conditions of environmental stress.

12-42 Sphingolipid metabolism in Arabidopsis

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Sphingolipids have been classically viewed as structural components of membranes but recent studies in yeast and mammals have revealed that sphingolipids and their metabolites are also dynamic regulators of multiple cellular processes. In plants, however, very little is currently known about the metabolism or function(s) of sphingolipids. In the course of our studies on the biosynthesis of polyunsaturated fatty acids, we identified paralogues of the "front-end" class of cytochrome b5-fusion fatty acid desaturases. These paralogues have been shown to desaturate sphingolipid long chain bases at the C8 position in a stereo-unselective manner. We have also identified the dihydrosphingosine desaturase that inserts a double bond at the C4 position to yield sphingosine. Sphingosine is the precursor of the apoptotic signalling molecule sphingosine-1-phosphate, which has recently been implicated in drought stress in plants. Intriguingly, unlike animals or yeast, plants display much greater heterogeneity of sphingolipids. We are currently dissecting the contributions of these modifications by a combination of functional characterisation and insertional mutagenesis. We have also identified several Arabidopsis genes involved in the C2 elongation of fatty acids. Fatty acid elongation is required for the synthesis of very long chain fatty acids, which are components of storage lipids, phospholipids, sphingolipids and waxes. Using tissue-specific gene silencing, we intend to determine the contribution of these different elongase components. We are also using biochemical complementation of yeast mutants defective in sphingolipid metabolism to assign function to candidate ORFs in the Arabidopsis genome.

12-43 Analysis of storage reserve breakdown in *ga1-3* and Paclobutrazol-treated Arabidopsis seeds

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The successful establishment of seedlings is dependent upon both germination and the mobilisation of seed storage reserves. We aim to understand the regulatory processes controlling storage reserve mobilisation during early post-germinative growth. Germination is known to be partly under the control of the endogenous phytohormones Abscisic Acid (ABA) and Gibberellic Acid (GA) which inhibit and promote germination respectively. A role for GA in the induction of storage reserve mobilisation in Arabidopsis has been investigated using the GA biosynthesis mutant *ga1-3*, and the GA biosynthesis inhibitor Paclobutrazol. We show that genes encoding key enzymes in lipid breakdown are not expressed in non-germinating intact seeds in GA-deficient backgrounds. However, removal of the seed coat from *ga1-3* seeds or wild-type seeds in the presence of Paclobutrazol, results in germination and greening of the embryo and the expression of genes essential for lipid breakdown. Anaylsis of protein and lipid breakdown confirms that reserve mobilisation is induced in a GA-deficient background only when the seed coat has been removed. We have previously shown that ABA does not block the mobilisation of storage reserves in Arabidopsis, as storage lipid breakdown is induced in seeds blocked from germination by ABA treatment. We conclude that although ABA-treated seeds and *ga1-3* or wild-type Paclobutrazol-treated seeds both fail to germinate, the events going on within the seeds are very different. While ABA-treated seeds are able to break down storage reserves, these processes are not induced in GA-deficient seeds unless the seed coat is removed.

12-44 CP12: A novel regulator of carbon metabolism

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The chloroplast protein, CP12, has been shown to bind to the Calvin cycle enzymes, glyceradehyde-3-phosphate dehydrogenase (GAPDH) and ribulose-5-phosphate kinase (PRKase), in vitro. Both of these enzymes are activated in the light by the ferredoxin/ thioredoxin system, and biochemical studies suggested that CP12 might be involved in the dark de-activation of GAPDH and PRKase. To determine the in vivo function of CP12 antisense transgenic plants containing reduced levels of this protein were produced. Light saturated photosynthetic carbon assimilation rates decreased and levels of sucrose and starch were also lower. Significant decreases in total biomass were observed, together with a dramatic reduction in total leaf area. Further phenotypic effects that could not be accounted for simply by the reductions in photosynthetic carbon assimilation were also observed in these plants. In the lines with the most severe phenotypes abnormal morphology of leaves and floral organs, greatly reduced fertility and markedly slower growth rates were observed. The nature of the CP12 antisense phenotype provides compelling evidence that this protein has an important role in regulating the allocation of carbon from the Calvin cycle. For this reason the CP12 antisense plants provide us with important and novel tools to investigate the regulation of chloroplast carbon metabolism. Our recent database searches indicate that in Arabidopsis thaliana there are three genes, located on chromosomes I, II and III. Two of these genes are very closely related at an amino acid sequence level (85% identity) and are known to be expressed, since ESTs are available.

12-45 Exclusive NH_4^+-transport by AMT-type ammonium transporters

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High affinity ammonium uptake and retrieval across plant and microbial plasma membranes is catalyzed by members of the ammonium transporter family (AMT/MEP/Rh). Six AMT-type transporters have been identified in the genome of Arabidopsis, while two ammonium transporters have been identified in tomato roots. These two tomato ammonium transporters were functionally expressed and analysed in Xenopus oocytes. Both transporters induced NH4+ specific, pH-independent currents. In uptake experiments using radiolabeled methylammonium, LeAMT1;2 mediated exclusive uptake of charged methylammonium, but not uncharged methylammonia. Both transporters bind NH4+ in a voltage-dependent manner and the binding site is localized 30% within the electric field across the membrane. Thus both transporters, which are 76% identical, encode NH4+ uniporters. In agreement with the transcript up-regulation of LeAMT1;1 at low external nitrogen availability, this transporter displays a high affinity (10 µM at -100 mV) towards NH4+. The affinity of LeAMT1;2, which is repressed at high nitrogen supply, is 6-fold lower. In conclusion, plant roots adjust their ammonium uptake by expressing transporters with different affinities and in most physiologic conditions these transporters import NH4+ along its electrochemical potential.

12-46 Sugars and phytohormones regulate seed germination and early seedling development via a complex signaling "network"

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Plants undergo profound metabolic and developmental transitions during seed germination and early seedling development. Regulation of these transitions and other processes occurs via a complex signaling "network" that receives input from several chemical signals. These chemical signals have long been known to include abscisic acid, which inhibits seed germination, as well as ethylene and gibberellin, which promote seed germination. Recent work by our lab and others has shown that soluble sugars also affect seed germination. Even low to moderate concentrations of exogenous sugars (e.g. 30 mM glucose) cause a significant delay in the rate of germination of wild-type Arabidopsis. This effect is not due to alterations in the osmotic potential of the media, as even substantially higher concentrations of sorbitol do not exert similar effects. Several lines of evidence also suggest that sugars and phytohormones "interact" in the regulation of seed germination. For example, several sugar-insensitive (*sis*) mutants of Arabidopsis also exhibit alterations in phytohormone response and/or metabolism. The *sis1* mutant is allelic to the ethylene constitutive response mutant, *ctr1*. The *sis4* mutant is allelic to the abscisic acid deficient mutant, *aba2*, and the *sis5* mutant is allelic to the abscisic acid insensitive mutant, *abi4*. Finally, the *sis2* mutant displays resistance to the gibberellin biosynthesis inhibitor paclobutrazol, suggesting that *sis2* may be defective in gibberellin response or metabolism. Findings that exogenous glucose greatly exacerbates the negative effects of paclobutrazol on seed germination also suggest "interactions" between sugar and phytohormone response pathways. Possible models for these interactions will be discussed.

12-47 Lipid metabolism and chloroplast biogenesis are severely impaired in the presence of exogenous glucose

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

12-48 Functional analysis of a *MYB* gene in Arabidopsis

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The fully sequenced Arabidopsis genome is predicted to encode at least 1,500 transcription factors. Of these, the MYB gene family is the largest with more than 180 members. Previous work has shown that MYB factors control diverse morphogenetic and metabolic processes, including the control of secondary metabolic pathways, growth regulators and epidermal cell shaping. Using genetic strategies, we have identified a gene trap transposon line with an insertion in the MYB gene designated atMyb68. This transposon inactivation produces the myb68 mutant which exhibits insensitivity to the phytohormone auxin in root culture, and which accumulates higher levels of soluble phenylpropanoid components in root tissues. This suggests that the Myb68 gene regulates the synthesis of certain phenylpropanoids which may be involved in cell wall modifications and/or lignification, and possibly the production of antimicrobial substances.

12-49 Increased epidermal wax accumulation mediated by a transcription factor in Arabidopsis

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In the course of our functional characterization of Arabidopsis transcription factors, we have identified a novel gene that plays a central role in the control of epidermal wax biosynthesis. Constitutive expression of WIN1, a member of the AP2/ERF family, causes a striking phenotype in transgenic Arabidopsis plants: wax levels are up to 10-fold higher in the leaf and at least two-fold higher in the stem, as compared to control plants. Detailed analysis indicates that wax constituents are differentially affected, and that build-up of long-chain alkanes accounts for most of the wax increase. Chemical analysis and transmission electron microscopy of WIN1 overexpressers indicate that large amounts of wax may be trapped in the cell wall under the cuticle. Higher levels of intermediary compounds, as well as higher expression of some pathway genes suggest that WIN1 may cause an increase in pathway flux, by inducing genes related to wax biosynthesis.

12-50 Isolation and characterization of a putative nitrate transporter, *ntl7*, in Arabidopsis

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CHL1 is a dual affinity nitrate transporter gene involved in both high- and low-affinity nitrate uptake. In Arabidopsis, there are about 52 CHL1 homologs which are temperately named as NTLs (Nitrate Transporter Like). In order to find out the in vivo functions of individual homolog, reverse genetic approach was used to identify mutants for each homolog. We isolated a T-DNA knock-out mutant, ntl7, with significant growth retardation phenotype. Gus activity assay demonstrates that NTL7 expresses in cotyledons and hydathodes of true leaves. Lots of droplets accumulate on cotyledon and the first 2 true leaves in this mutant, which is not observed in wild type plants. Further investigation is needed to elucidate whether this phenomenon is a result of nitrate concentration affecting water potential. The copy numbers of T-DNA insertion in ntl7 mutants are determined by southern analysis and the segregation ratio by kanamycin selection. Lost-of-function of the target gene will be confirmed by the analysis of Northern, Western blots, and nitrate uptake/transport activity. Analysis of this mutant will help us to understand how the nitrate transporters affect on plant growth in details.

12-51 Studies on the enzymatic systems of inorganic pyrophosphate and polyphosphate methabolism in *Arabidopsis thaliana*

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Inorganic pyrophosphate (PPi) is an abundant by-product of metabolism whose removal is essential to shift the equilibrium of anabolic reactions towards biosynthesis. Nine genes encoding PPi-hydrolysing enzymes (inorganic pyrophosphatases, EC 3.6.1.1) of the two families described so far -namely soluble (sPPases, hydrolyse PPi yielding heat) and membrane-bound (V-PPases, couple PPi hydrolysis to proton movement across intracellular membranes)- occur in the genome of *A. thaliana*. Among the six sPPase-encoding *ppa* genes, one encodes a chloroplastic eukaryotic-type sPPase precursor expressed in green tissues but not in roots whereas a set of five closely-related paralogous *ppa* genes located in different chromosomes should encode a family of bacterial-type sPPases, one of them being probably a mitochondrial precursor. Since all microalgal and plant chloroplastic sPPases that we have studied so far are nuclear-encoded eukaryotic-type proteins, it is likely that the ancestral cyanobacterial-like sPPase was functionally substituted by the sPPase of the eukaryotic host cell during the early evolutionary processes that gave rise to photosynthetic plastids. Three genes *(AVP1, AVP2 and AVP3)* code for V-PPases in the genome of *A. thaliana*. AVP2 and AVP3 (potassium-independent pumps) are closely related whereas AVP1 (a potassium-stimulated pump), although having typical V-PPases motives, only shows an overall 36% homology with the other two proteins. AVP1 can complement functionally the cytosolic sPPase of yeast. This is the first direct evidence that AVP1 can mediate net hydrolysis of PPi *in vivo*. Expression studies with specific probes for *AVP1* and *AVP2* have been performed in different tissues of *A. thaliana* under salt-stress

12-52 The biosynthetic pathway of vitamin C in higher plants

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Vitamin C (L-ascorbic acid) in fruits and vegetables is an extremely important vitamin for human nutrition. Despite the diverse biological functions of vitamin C, very limited information is available about the pathway(s) leading to its biosynthesis in many plants. The described vitamin C biosynthetic pathway proposed in higher plants uses L-galactose as the immediate precursor of the key intermediate L-galactono-1,4-lactone. Unquestioned support for this pathway was obtained after the characterization of an Arabidopsis AA-deficient mutant (vtc), which encodes for GDP-mannose pyrophosphorylase. However, Arabidopsis cell suspensions can synthesize L-ascorbic acid from a number of precursors that include methyl-D-galacturonic acid, what suggests that alternative routes also exist in Arabidopsis. We have identified and isolated a key gene of this pathway, GalUR, encoding a D-galacturonic acid reductase from strawberry fruit, an organ that produces high levels of vitamin C. Overexpression of GalUR in Arabidopsis increased the vitamin C content 2-3 fold, demonstrating the feasibility of engineering increased vitamin C levels.

W01 Nitrogen networks in plants

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The Arabidopsis genome project has uncovered a large set of genes involved in nitrogen uptake, metabolism, and allocation (600+). Expression studies on a small subset showed that N-status regulates the transcription of many of these genes. Proposed N-signals include nitrate, ammonium, glutamate, glutamine, and C:N balance. At present, there is little or no understanding of the regulatory molecules or networks involved in signaling N-status and integrating N metabolism with plant growth. We are using expression arrays to identify circuits of genes regulated by N-status (inorganic-N and organic-N). We are using bioinformatic analysis to identify co-regulated genes and N-responsive cis-elements. We also aim to determine the function of key genes we identify by defining the phenotype of mutants lacking each gene and by determining the properties of the expressed proteins. A computer cluster will store the large amounts of data generated in this project. A publicly accessible Web page (http://www.nyu.edu/fas/biology/n2010) will include access to expression databases, gene identification information, and all software developed in the proposed project. The new software developed includes new clustering algorithms, cis-search programs, and a bioinformatic pathway tool called "PathExplore" that can be used to query microarray expression datasets for expression of genes in common pathways. The results should substantially advance our understanding of nitrogen metabolism in the context of plant growth, as well as provide new insights into our understanding of complex regulatory networks. Given the central role of nitrogen availability and metabolism in crop productivity, these results should also have broad agricultural impacts.

W02 The Arabidopsis RPM1 disease resistance signaling network

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A detailed understanding of the molecular events leading to plant disease resistance is of practical importance in agriculture, and presents extremely interesting basic research challenges. Genetic control of plant disease resistance reactions is commonly determined by specific interactions between a particular allele of a pathogen *avr* (*avirulence*) gene locus and an allele of the corresponding plant disease resistance (R) locus. There are ~175 Arabidopsis R gene homologues whose deduced proteins share a nucleotide binding site and a run of imperfect Leucine Rich Repeats (NB-LRRs). RPM1 acts on the inside of the cell plasma membrane to condition resistance to strains of phytopathogenic *Pseudomonas syringae* which carry the corresponding a*vrRpm1* gene, and to another *P. syringae* gene, called *avrB*, which has no obvious homology with *avrRpm1*. Thus, *RPM1* encodes a "dual specificity". Engagement of NB-LRR proteins initiates signal transduction events culminating in a halt of pathogen growth. Isolation of R genes now focuses attention on the biochemistry and cell biology of how NB-LRR proteins interact with other plant cellular machinery to cause disease resistance. Our 2010 project takes complementary biochemical and genetic approaches to dissect the RPM1-dependent signaling network. We have generated a large set of new tools, and identified an expanding set of genes, which function in the RPM1 signaling network, and in responses dependent on other *R* genes. Thus, our data are at once enlightening with respect to RPM1 in particular and *R* gene signaling in general.

W03 Expression profiling of plant disease resistance pathways

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Arabidopsis exhibits all of the major kinds of defense responses present in other plants. Although a relatively large number of Arabidopsis defense-related genes have been identified, progress in developing overall explanatory models of plant-pathogen interactions is currently limited by two major factors. First, most of the phenotypic tests that have been used to characterize pathogen-host interactions do not have sufficient discriminatory power to assign defense-related genes to specific signal response pathways. To circumvent this limitation, large-scale transcript profiling will be carried out on selected Arabidopsis defense-related mutants. Combining genetic epistasis analysis with genomic technologies should lead to the development of a comprehensive model for plant defense responses. The second factor limiting the understanding of the plant defense response is that it is simply not possible to analyze the overwhelming volume of current data using conventional methods. The large volume of microarray data that will be generated in the near future compounds this problem. To help mitigate the problems associated with the analysis of large data sets, a web-accessible plant-microbe interaction database (PMIDB) will be created to provide a common repository and standardized format for experimental data. The specific aims of the project are to: (1) Use transcript-profiling to identify Arabidopsis defense-related genes and construct a custom microarray (pathoarray) enriched for defense-related genes; (2) Use the pathoarrays from Aim 1 and Arabidopsis defense-related mutants to define the expression signatures resulting from the activation of defense pathways; (3) Create a web-accessible plant-microbe interaction database (PMIDB; http:// genetics.mgh.harvard.
edu/ausubelweb/nsf2010/NSF_2010.html), in the next four years.

W04 A sequence-indexed library of insertion mutations in the Arabidopsis genome

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With the availability of the entire Arabidopsis genome sequence, the next major challenge is to uncover the functions of the more than 25,500 genes in this reference plant. Given the scope of the NSF 2010 program, "to identify the function of all Arabidopsis genes in the next decade", efficient and cost effective approaches are necessary to identify mutations for all genes. The goal of this program is to create a sequence-indexed library of mutations in the Arabidopsis genome. The Salk Institute Genome Analysis Laboratory (http://signal.salk.edu) is using high-throughput genome sequencing methods to identify the sites of insertion of Agrobacterium T-DNA in the Arabidopsis genome. T-DNA-transformed plants from the Alonso/Crosby/Ecker collection are grown, genomic DNA is prepared, and T-DNA-flanking plant DNA are recovered and sequenced. Insertion site sequences are aligned with the Arabidopsis genome sequence and gene annotation is added. The data are made available with daily updates via a web accessible graphical interface- Arabidopsis Gene Mapping Tool (http://signal.salk.edu/cgi-bin/tdnaexpress). All DNA sequences are deposited into GenBank and also provided to The Arabidopsis Information Resource. Thus far, seeds from over 50,000 sequenced T-DNA insertion lines have been deposited with the Arabidopsis Biological Resource Center. The creation of a text and sequence searchable mutation database containing the insertion site sequence information for 150,000 T-DNA lines and the availability of the corresponding seeds in public stock centers will provide researchers with ready access to mutants in their genes of interest, allowing the testing of hypotheses about gene function at an unprecedented rate.

W05 Identifying clients of 14-3-3 phosphoregulation

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The 14-3-3 proteins have emerged as critical regulators of diverse cellular processes that utilize phosphorylation signaling. Phosphorylation alone is often not sufficient to cause a change in the activity state of a target protein, and the subsequent binding of 14-3-3s to the phosphorylated target is required to complete the signal-induced transition. This fundamental property of 14-3-3s appears to be intrinsically necessary for regulatory events all eukaryotes, and especially in plants. The primary goal of this project is to identify in the Arabidopsis genome client proteins that possess 14-3-3 docking sites and then assay the interaction strength of the potential docking site against the different members of the Arabidopsis 14-3-3 family. The result should be a tested, predictive algorithm for determining of the spectrum of 14-3-3 interactions that should be expected for each potential client within the proteome. These data, combined with emerging data from 14-3-3 knockouts and 14-3-3 family member expression patterns, should provide key insights into the potential regulatory events impacted by 14-3-3s, and a central, fully characterized database for predicting 14-3-3 mediated signaling in diverse pathways. By highlighting potential regulatory sites across diverse gene families and metabolic pathways, this project can provide an integrating effect on the many projects identifying specific functional aspects of Arabidopsis gene families. As genes are functionally characterized, the 14-3-3 client database will provide immediate predictions of phosphorylation and 14-3-3 dependent regulation that will accelerate the understanding of the regulation of many gene families and metabolic and signaling pathways.

W06 Developing paradigms for functional genomics of protein kinases and phosphoproteins using the CDPK superfamily

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Protein phosphorylation is a major mode of regulation of metabolism, gene expression, and cellular architecture in eukaryotic cells, and defining phosphorylation-based regulatory networks is essential for understanding the function of the Arabidopsis genome. The goal of this project is to define phosphorylation networks that are related to the function of calcium-dependent protein kinases (CDPKs) and four closely related families; CDPK-related kinases (CRKs), phosphoenolpyruvate carboxylase kinases (PPCKs), PPCK-related kinases (PEPRKs), and SNF-1 related kinases (SnRKs). These 88 kinases, which represent about 9% of all the protein kinases in the Arabidopsis genome, are involved in all aspects of plant development and physiology and participate in the coupling of cellular responses to environmental and developmental signals. A list of these protein kinases including gene identification numbers is available at http://www.arabidopsis.org, and further information including links to database records is available at http://plantsp.sdsc.edu. This research will investigate the function of 64 members of these families through determination of the subcelular location of each kinase and identification of downstream targets and other proteins with which the kinases associate. This information will give insight into the physiological roles of each kinase by identifying signaling networks in which each participates. This research will also determine the target sequences in substrate proteins that are phosphorylated by each kinase, and these results will contribute to understanding the overlap in kinase function and cross-talk between signaling pathways. The results of this work will be made available on a yearly basis at the two URLs given above.

W07 Analysis of two-component signaling elements from Arabidopsis

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Two-component systems are the primary means by which bacteria sense and respond to environmental stimuli. These systems are comprised of a number of distinct elements, namely histidine kinases, response regulators and in the case of phosphorelays, histidine phosphotransfer proteins (HPts). Genes encoding similar proteins to each of these elements have been identified in Arabidopsis, and for the majority of the 35 such genes no function has yet been definitively ascribed. A combination of gene knockouts and inducible overexpression will be used to assess the roles of these genes in plant growth and development. The mutant plant lines will be characterized in terms of their response to biotic and abiotic factors such as hormones, light, and osmotic stress, and for their pattern of gene expression. Where in the plants these genes are expressed will be determined using a combination of GUS fusions and in situ RNA analysis. The location of the cognate proteins within the cell will also be delineated. To facilitate this localization, a series of 10 monoclonal antibodies will be generated to marker proteins, each of which resides on a distinct membrane. Protein complexes from Arabidopsis will be purified and analyzed to determine the interactions among these elements and to identify novel interacting proteins. Together, these studies will illuminate the signaling pathways in which each of these Arabidopsis two-component signaling elements function and how they interact to control plant growth and development. The data from these studies will be deposited on a publicly accessible web site (http://www.bio.unc.edu/research/two-component/).

W08 Comprehensive functional analysis of the Arabidopsis RCD gene family

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Apocarotenoids are a diverse set of plant secondary metabolites derived from carotenoid breakdown. They have significant roles as developmental and environmental response signals as well as contributing to flavor and nutritional quality. A critical advance in elucidating apocarotenoid synthesis occurred when the first gene encoding a carotenoid dioxygenase, maize Vp14, was cloned. Vp14 encodes the limiting enzyme in abscisic acid synthesis, 9-cis-epoxycarotenoid dioxygenase (NCED). Arabidopsis contains nine members of a gene family encoding enzymes related to NCED, referred to hereafter as RCDs (Related to Carotenoid Dioxygenase). Only a subset of the family is directly involved in abscisic acid synthesis and the other members of the family likely encode enzymes that metabolize a range of carotenoids to multiple volatile and non-volatile apocarotenoids. One of the family members, RCD1, does indeed cleave multiple carotenoid substrates at a different position than NCED, supporting this hypothesis. Thus, the RCD family exhibits variation both in carotenoid substrates and position of cleavage. We will establish the functions of the complete Arabidopsis RCD gene family. We have identified knock-out mutants in every RCD gene and will construct double and triple knockout combinations, as appropriate. Using bacterial expression and in vitro assays, the substrate specificities and products of each member of the RCD enzyme family will be determined and metabolic profiles of each mutant will be quantified. Mutants will also be examined for phenotypic alterations associated with altered apocarotenoid profiles.
Information on the RCD gene family and characterization of individual gene products may be obtained at http://www.hos.ufl.edu/kleeweb/2010RCDprogram.htm

W09 Phenylpropanoid pathway networks: An integrated approach to establishing protein/enzyme function in Arabidopsis and their associated networks

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The goal of this project is to establish the physiological function of 248 Arabidopsis enzymes and proteins presumed to be involved in various networks of phenylpropanoid-acetate metabolism. There are two main objectives: identifying networks associated with phenylpropanoid coupling/ polymerization, including how these enzymes/proteins function. The second objective is to precisely identify the different networks that exist in Arabidopsis that are involved in the conversion of phenylalanine through to the monolignols. In both objectives, functions will be demonstrated in vitro, and by demonstrating temporal and spatial correlation with the segments of the metabolic pathway networks involved. This work will thus define the organization of the various phenylpropanoid radical-radical coupling and related metabolic processes in Arabidopsis through its entire life cycle. For enzymes/genes chosen that are ultimately not involved in these pathways, it is considered that metabolite profiling will provide a clue as to function, and this will then be examined also. In addition to lignification, this study will shed important light on other highly regulated radical-radical phenolic coupling systems in vivo. Progress towards these goals, in the first phase of the study (Year 1), is summarized.

W10 Determination of the biological functions of the NPH3/RPT2 family

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Developmental plasticity is a key feature for the evolutionary success of sessile organisms since responses to environmental cues have to be precise and flexible to maximize their fitness value. The assembly of multimolecular complexes on scaffold proteins increases the speed and selectivity for specific signaling pathways utilizing signaling molecules otherwise common to multiple pathways. The NPH3 and RPT2 proteins of Arabidopsis are members of a novel plant-specific superfamily hypothesized to represent scaffold proteins. As part of NSF Arabidopsis 2010 Project, we are determining the biological functions of all members of the NPH3/RPT2 gene family (32 members). We are generating mRNA and protein expression profiles as well as loss- and gain-of-function mutants for each member. We will use these tools to design targeted physiological and developmental analyses to determine biological functions. A high-throughput cDNA-AFLP-based approach is being developed to examine mRNA profiles under a large number of conditions. *In silico* analysis for a minimal number of primer pairs giving gene-specific transcript-derived fragments based on fragment size and selective nucleotides is underway. We have identified potential loss-of-function mutant lines for many members of the family, and are currently verifying these lines and generating near-isogenic stocks. Gain-of-function alleles are being generated transgenically by driving expression of family members from their native promoters fused to 4 transcriptional enhancer elements from the CaMV 35S promoter. We hypothesize that this approach will result in overexpression of the genes in the correct temporal and spatial fashion, allowing for clearer interpretation of phenotypes relative to loss-of-function alleles.

W11 A systematic approach to automated production of Recombinant Inbred Lines

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The goal of this project is to develop a resource for the scientific community that promotes expanded use of natural genetic variation. At least four new sets of mapped Recombinant Inbred Lines (RILs) will be produced. Potential pairs of parents from hundreds of available wild type accessions will be systematically screened for genetic and phenotypic variance and pairwise distances. The chosen parents will include wild lines not previously exploited. Simple Sequence Length DNA Polymorphisms will be used as much as possible for mapping. Ninety-five individuals, from sets of 400 RI lines will be mapped at one hundred loci, generating a map with a density of approximately 6 centiMorgans. Seeds for all RILs will be available through the ABRC and maps and mapping data will be available online through TAIR by January 2004. Conventional genetics used in a small number of laboratory strains of Arabidopsis, will not be able to reach the 2010 Project goal of complete knowledge of every plant gene function. This project will provide a way to map and identify genes in local wild populations of Arabidopsis that are important for adaptation to many different environments and are the result of many different evolutionary histories. These different environments will include different soil types, day lengths, pest populations, moisture levels, temperatures, and other factors. This will eventually lead to a better understanding of important adaptations to incorporate into the world crops.

W12 Essential gene functions in Arabidopsis seed development

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The SeedGenes Project (www.seedgenes.org) focuses on genes with essential functions in growth and development that give a visible seed phenotype when disrupted by mutation. Arabidopsis appears to contain 500 to 750 such *EMB* genes required for seed development and another 200 genes required for seed pigmentation. Our goal is to co-ordinate the collection, analysis, and presentation of information on these genes based on cloning of mutant alleles. Project objectives are to approach saturation for cloned *EMB* and seed pigment genes; standardize phenotypic characterization of the corresponding mutants; understand the functions of these genes in growth and development; determine through expression studies and comparative sequence analysis why these genes are essential; and integrate this information into a project database accessible through the web. The first release of this database (March 2002) contains information on 100 genes and their knockout phenotypes. Included are 60 genes identified at Syngenta and another 40 genes described in the literature. Additional mutants and genes will be added to the project database at scheduled intervals over the next several years. The final goal is to present information on 500 mutants defective in 300 different EMB genes and another 100 mutants defective in 75 genes required for seed pigmentation. This project was made possible through a large-scale insertional mutagenesis program initiated 5 years ago at Syngenta (North Carolina) in collaboration with the Meinke laboratory (OSU). Initial results are described in Genetics 159: 1751-1763 (2001). The Virginia Bioinformatics Institute (VBI) is coordinating gene expression, database, and web site functions.

W13 Pre-mRNA splicing signals in Arabidopsis

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Splicing enhancers are known to function in splice site selection and to contribute to the regulation of alternative splicing in animals. While plant splicing enhancers are likely to play a similar role, they have not yet been described in detail. A database of Arabidopsis exons and introns has been constructed and is being used to identify candidate splicing enhancer sequences. The role of these sequences and sequences from genes that are known to be alternatively spliced will be tested in transgenic Arabidopsis using a splicing reporter construct. Specifically, the activity of these enhancers will be examined in transgenes that depend on exon inclusion for GUS expression. This will include analysis of the tissue-specificity of splicing enhancer activity and should provide an extensive database of information about the role of particular sequences in promoting splicing.
Ultimately, this project will lead to improved gene finding and gene annotation, both directly as improved gene annotations (http://www.tigr.org/tdb/ath1/htmls/ath1.html) and as improved performance by the GlimmerM server (http://www.tigr.org/softlab/glimmerm/). Transgenic lines carrying splicing reporter genes with defined candidate splicing enhancer sequences will be available through the ABRC. These lines may be useful as reporters of regulated splicing or as tissue-specific markers in their own right. Information about GUS expression in these lines, including written descriptions of all expression patterns and images of typical and selected patterns, will be available on our web site (http://www.tigr.org/2010-splicing/).

W14 Development of laser-capture microdissection for plant tissues

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Laser-capture microdissection (LCM) is a technique originally developed for animal tissues, whereby individual cells are harvested by tacking them to a plastic film with a low-power infrared laser that can be aimed at single cells while viewing the tissue slice through a microscope. Using this method, it is possible to recover specific cell types or developmental stages from complex tissues consisting of many cell types. Cells recovered in this manner can be analyzed with regard to gene expression profiles, protein profiles, and other properties. We are testing a variety of tissue preparation and RNA/DNA/protein recovery methods to adapt LCM to plants.
Tissues with simple organizations and relatively large cells are being tested first, and more complex tissues with smaller cells will follow. Accordingly, we developed initial protocols for isolation of bundle sheath and mesophyll cells from maize leaves and of mesophyll cells from Arabidopsis leaves. We are now developing protocols for smaller cell types in developing tissues of Arabidopsis and maize, including meristematic, provascular and procambial cells. Protocols optimized for Arabidopsis and maize tissues will be tested and adapted for a wide range of plant species. We are at the same time optimizing protocols for recovery of RNA of sufficient length and quality for expression profiling. These properties are highly dependent on the combination of tissue fixation and RNA extraction protocols. Optimized protocols will be provided to the biological community at the website http://plantgenomics.biology.yale.edu.

W15 The genealogy of *Arabidopsis thaliana*

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High levels of linkage disequilibrium and polymorphism, combined with the availability of inbred lines, suggest that a haplotype map of *Arabidopsis thaliana* could be extremely useful for mapping loci responsible for natural variation. We will construct such a map by sequencing approximately 2000 short (500-700 bp) fragments distributed throughout the genome in a sample of 96 accessions (one 96-well plate). This means on the order of four fragments per cM, or one fragment every 50 kb. We will also develop a database and bioinformatics tools to make the data available and useful.
The 96 accessions will be chosen as follows: The first half of the plate will consist of stock center accessions, chosen so as to include most accessions that are currently being used in developing recombinant inbred lines (RILs) or in SNP-detection projects. The reminder of the sample will consist of a stratified sample of several freshly collected accessions from each of a number of populations in Europe and the USA. The purpose of this is to get further insight into the population structure of A. thaliana. Seeds from the chosen accessions will be made available through the stock centers.

W16 Functional genomics of the Arabidopsis ß-glucosidase and ß-galactosidase gene families

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O-Glycoside hydrolases (EC 3.2.1.-) catalyze the cleavage of chemical bonds between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety. Within the scope of the 2010 Program to identify the function of all *Arabidopsis thaliana* genes, this collaborative research will focus on approximately 65 members of two related families of glycoside hydrolases (http://www.biol.vt.edu/faculty/esen/glycosidase.lab.html, http://www.biology.uiowa.edu/arabidopsis/ and http://afmb.cnrs-mrs.fr/CAZY/index.html). Family 1 includes β-glucosidases (EC 3.2.1.21) and myrosinases (EC 3.2.3.1), which function in higher plants in chemical defense against herbivores and pathogens, lignin biosynthesis, and plant growth and development. Family 35 contains the β-galactosidases (EC 3.2.1.23), which play key roles in fruit ripening, flower senescence, mobilization of carbohydrate reserves, and galactolipid turnover. The purpose of this multidisciplinary research is to assign biological functions to these Arabidopsis hydrolases. After using phylogenetic analysis to identify subfamilies that contain closely related enzymes, cDNAs encoding the target hydrolases will be obtained and overexpressed in heterologous host cells. After purification, the biological function of each hydrolase will be determined by measuring its enzymatic activity toward a wide range of natural glycosidic substrates isolated primarily from Arabidopsis and related crucifers. In parallel studies, three-dimensional structures of selected subfamily representatives will be determined by homology modeling and x-ray diffraction, providing novel insights into how these hydrolases recognize and bind their substrates. This information will be of paramount importance in future research to alter the substrate specificity of Family 1 and Family 35 hydrolases for biotechnological purposes, including biomass conversion and improvements in anti-herbivore defenses and fruit ripening.

W17 From seed to seed: Genome-wide expression analysis of Arabidopsis throughout its life cycle

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Arabidopsis thaliana is a plant model organism that has become a major research tool for plant Biologists. A primary motivation for the sequencing of Arabidopsis was that it would serve as a reference for other plant genomes. For this to be realized the functions of a large number of unknown and hypothetical genes must be deduced and a cross-reference between genes in Arabidopsis and other plant species provided. Ultimately, the development of the data resources will lead to a better understanding of gene expressions and function in plants.
Predicted genes will be amplified and used to construct a whole genome microarray that will be used to look at patterns of gene express through the plant life cycle. Expressions data can help provide both functional assignments for genes and used in identifying metabolic pathways. The data will be analyzed using a suite of clustering and pattern recognition tool. A number of databases will be combined and integrated to provide a web-based view of gene expression and identification in Arabidopsis.

W18 Functional genomics of Arabidopsis P450s

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This NSF2010 project is aimed at functionally defining the expression patterns and substrate reactivities of the 272 members of the cytochrome P450 monooxygenase (P450) gene superfamily that represents 0.6% of the Arabidopsis genome. P450 proteins encoded by these genes mediate oxidative transformations in an array of biosynthetic and detoxicative pathways essential for plant growth. Because of the wide diversity of metabolic processes in which they participate, they serve as downstream reporters for the direct activation of many different pathways responding to chemical, developmental and environmental cues. Extensive divergence of catalytic site residues has resulted in a degree of structural divergence that has confounded the assignment of function to individual P450 sequences. We are furthering their functional definition by creating P450 microarrays for expression analysis of tissues at different developmental stages and treated with different chemicals and environmental conditions (UV damage, pathogen attack, insect attack, etc.). Collations of these response patterns with respect to plant biochemical pathways will provide the basis for assignment of individual P450 sequences to pathways. Overexpression of a subset of these P450s in yeast and baculovirus systems and incorporation into membrane-scaffolding protein complexes suitable for high-throughput screening of substrate reactivities will provide the basis for assigning more definitive function(s). These results should help explain this plant's biochemical responses to a variety of stresses and provide functional expression tools needed for assessment of other membrane proteins. Data on the organization of the 272 P450 genes, alignments of available ESTs and some defined functions have been made available at http://arabidopsis-P450.biotec.uiuc.edu.

W19 Identification of the function of a family of putative glycosyltransferases

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The goal of this project is to determine the biological function of genes that encode six families of structurally related proteins, called the cellulose synthase like (CSL) proteins. Based on the sequence similarity to cellulose synthase, it is hypothesized that the CSL genes encode processive glycosyltransferases that may catalyze the synthesis of some of the non-cellulosic polymers that comprise plant cell walls and other polysaccharides such as stylar secretions and mucilage, or the glycosyl residues on arabinogalactan proteins. The technical approach that will be used to determine the function of the CSL genes exploits the recently completed full genome sequence of Arabidopsis to facilitate directed genetic analysis of the genes. Most of the CSL genes will be inactivated by insertional mutagenesis. In addition, the expression of the CSL genes will be altered by producing transgenic plants that have increased or decreased accumulation of mRNA for the CSL genes. The effects of the mutations and transgenic events on the growth and development of the plants, and on plant polysaccharide composition will be analyzed in order to associate each CSL gene with a specific biological role and enzyme of known catalytic activity. A list of the accession numbers of the genes to be analyzed during this project can be found at
http://www.arabidopsis.org/info/2010_projects/index.html. Information about the gene families can be found at http://cellwall.stanford.edu/cesa/index.shtml.

W20 Arabidopsis 2010. The endgame for reverse genetics: Isolation and distribution of a knockout mutant for every gene in Arabidopsis

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We have been funded to develop and implement novel methods for genotyping Arabidopsis mutants. Our ultimate goal is to provide the Arabidopsis community with a knock-out allele of every gene in the genome through the use of both T-DNA insertional mutations and point mutations induced by a chemical mutagen. Our method utlizes the maskless array synthesizer (MAS), which is a novel instrument for fabricating oligonucleotide arrays developed recently at the University of Wisconsin. The MAS uses photolithography chemistry similar to that pioneered by Affymetrix with one important difference. In the place of chromium masks, which are expensive and time consuming to produce, the MAS uses a digital micromirror array (DMD) to direct and regulate 786,000 independent light beams on a glass surface. Computer-controlled virtual *masks* are created via computer software each time a new oligo array is needed. Thus, the MAS creates glass microscope slides containing 786,000 unique oligonucleotides of any length, and a different set of such oligonucleotides can be made every 2 hours.
We are using this flexible platform of high density oligonucleotide arrays to (1) develop a less expensive and more rapid means of obtaining hundreds of thousands of flanking sequences for T-DNA insertions, and (2) identify plants containing specific stop codon mutations in a chemically mutagenized population. Although this project is focused on Arabidopsis, the technologies are applicable to other any sequenced species as well.

W21 Plant peroxisomal biogenesis - Sorting/function of membrane proteins and peroxins

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Peroxisomes are ubiquitous subcellular organelles that possess a diverse array of enzymes and other proteins that vary in response with developmental stage, metabolic change and demands, toxic ROSs, environmental cues, etc. The overall goal of this project is to identify Arabidopsis genes coding for peroxisomal membrane proteins (PMPs) involved in the biogenesis and functioning of peroxisomes, and then to use these and related data to elucidate function(s) of the gene products. "Peroxin" genes code for a set of proteins that participate specifically in peroxisomal biogenesis. Most peroxins are PMPs. To date, 15 Arabidopsis orthologs of 23 eukaryotic peroxin genes have been identified, but a function is known for only 4 of these 15 orthologs. Public access to this information is available at http://lsweb.la.asu.edu/rtrelease. The function(s) of peroxins and selected non-peroxin PMPs will be determined experimentally through a multi-pronged approach, i.e., elucidation of their subcellular localization, intracellular sorting pathways, and molecular sorting signals in suspension cells produced from wild type and mutant plants, through RNA/EST interactions on microarray chips, and via analyses of available knockout plants. The research is expected to elucidate the function of 10-15 genes related to the biogenesis and functioning of Arabidopsis peroxisomes. Since peroxisomal mutations are lethal in humans, and peroxisomes are essential for seedling establishment and photo-autotrophic growth of oilseed crop plants, the knowledge obtained likely will be applied to biotechnological improvements of agriculturally-important crop plants, and hopefully will speed therapeutic resolution of peroxisomal diseases in human infants.

W22 Functional analysis of the ubiquitin-protein ligase (E3) families in Arabidopsis

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The ubiquitin (Ub)/26S proteasome proteolytic pathway plays an integral role in the growth, development, homeostasis, and defense of plants by selectively removing abnormal polypeptides and short-lived regulatory proteins. Proteins are tagged with Ubs; substrates are then degraded by the 26S proteasome. The Ub-protein ligases (or E3s) determine the specificity of Ub conjugation and hence control selectivity. Their importance in plants is best demonstrated by the fact that the Arabidopsis genome encodes over 900 different E3s. Preliminary genetic analyses of just a few have identified important roles for E3s in hormone and stress responses, light signaling, circadian rhythms, enzymatic regulation, and pattern formation. To help define the depth and breath of ubiquitination in plants, this project will analyze the Arabidopsis E3 families. Bioinformatic approaches will be used to assemble the E3s into subfamilies. For representative examples, protein interaction techniques will assign each with appropriate accessory factors and biochemical assays will confirm that each has Ub-ligase activity. Their expression patterns and locations will be examined by microarrays and by GFP-E3 fusions. Yeast two-hybrid and mass spectrometric techniques will identify substrates. Functions will be explored by phenotypic analysis of appropriate mutants. Information will be released to the scientific community. The results generated will form a framework for understanding E3 diversity, help reveal specific functions, and will develop a database of proteins whose abundance is affected by Ub-mediated proteolysis. The project will provide information on an important cluster of Arabidopsis genes/proteins that ultimately can be used to devise new strategies to alter proteolysis.

W23 Bioluminescence Resonance Energy Transfer (BRET)- A tool to explore protein-protein Interactions in Arabidopsis

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We are developing a novel optical technique, termed Bioluminescence Resonance Energy Transfer (BRET), as a resource for the plant functional genomics community. BRET has shown promise as a tool to chart the physical contacts between specific cellular proteins, and their time-resolved interactions, in living plant tissue and in real time. BRET is a form of radiation-free energy transfer that can occur when two compatible optical probes are brought into molecular proximity. In detail, to probe for a protein-protein interaction between two given partner proteins, the two proteins are genetically fused to a blue light emitting luciferase and to a blue light absorbing yellow fluorescent protein. If the two hybrid proteins interact, the excitation energy of the luciferase may be transferred to the fluorescent protein, resulting in an easily detected yellow-shift in the luminescence spectrum. The first goal of this project is to construct tools, such as cloning vectors, for the expression of BRET hybrid proteins. Second, instrumentation and experimental protocols are being optimized for time-resolved BRET data acquisition. Moreover, as a prototype example of the efficacy of BRET for research in plants, BRET is being applied to probe the genetic networks that mediate the control of nuclear gene expression by light and the circadian clock.

W24 Functional analysis of the Arabidopsis Yellow Stripe-Like (YSL) family: Heavy metal transport and partitioning via metal-nicotianamine complexes

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The regulation of metal distribution within plants is a complex and relatively poorly understood process. The *YS1* gene of maize, which encodes a protein of the oligopeptide transporter (OPT) family, has been identified by our laboratory as an iron transporter in maize. YS1 performs phytosiderophore-mediated Fe3+ uptake from soil through a specialized mechanism that is unique to the grasses. We have recently identified eight *YS-Like* genes (*YSL1-8*) in *Arabidopsis thaliana* with strong sequence similarity to Ys1of maize. Arabidopsis is a non-grass and thus does not synthesize PS or transport PS-bound iron. However, Arabidopsis does make nicotianamine (NA), the biosynthetic precursor to PS. We hypothesize that NA acts as a metal chelator that efficiently and appropriately sequesters Fe, Zn, Cu, and Mn within the plant. We further propose that the YSL proteins act as membrane transporters that specifically transport NA-metal complexes into appropriate target cells.
We will form a comprehensive description of the function of each YSL protein that will answer these questions: Which metals do YSL proteins transport? Do these metals have to be provided as NA complexes? Which cells express each YSL and are therefore involved in transporting these metals? Which nutrient conditions affect expression of each *YSL* family member? In what way(s) does abolishing specific *YSL* genes affect the pattern of metal ion distribution in the plant? By integrating this specific information for all eight *YSL* genes, our understanding of the metal ion allocation mechanisms used by plants will be greatly improved.

W25 Genetic and physiological characterization of Arabidopsis plasma membrane H^+-ATPase mutants

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We are using a reverse genetic strategy to study the Arabidopsis plasma membrane H^+-ATPase (*AHA*) gene family. Expressed in different tissues and coded by a gene family with twelve members, the proton pumps contribute to a variety of processes such as cellular homeostasis, mineral and metabolite transport, morphogenesis and responses to the environment. We have identified twenty-nine T-DNA insertion lines, including ten of twelve *AHA* gene family members. Ongoing work includes: isolating, outcrossing and preparing *aha* lines for analysis and distribution; performing crosses to create multiple insert lines in order to uncover genetic redundancy; testing single and multiple mutant lines for conditional phenotypes by comparing mutants to wild type under conditions designed to reveal differences in development, responses to environmental stimuli, nutrient uptake, etc. In addition to the core work on the *AHAs*, we are electronically publishing a database of experimental conditions (http://thale.biol.wwu.edu). Reverse genetic strategies are often hindered when mutant phenotypes are not apparent. When the gene is a member of a gene family, or when the function of the gene is uncertain, a broad-spectrum panel of assays may serve to uncover conditional phenotypes.
The twenty-nine *aha* mutant alleles provide the raw materials for progress into determining the function of the *AHA* gene family. The WEB site is an ongoing project that will provide members of the Arabidopsis community the ability to quickly establish a broad range of conditional assays. Project 2010 support at a predominantly undergraduate institution provides a means of integrating genomic scale research with undergraduate training.

W26 Functional analysis of Arabidopsis subtilisin-like seine proteases

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This project aims at the functional analysis of the subtilisin-like serine protease gene family in Arabidopsis thaliana, which includes 57 members. Known eukaryotic subtilases (mammalian in particular), activate through specific cleavage prohormones or proreceptors involved in the control of various developmental or metabolic processes. Several plant subtilases have been isolated that are potentially involved in signalling processes triggered by environmental stimuli (wounding, pathogen attack) or related to developmental processes. Through the analysis of a corresponding mutant, the Arabidopsis SDD1 subtilases was shown to fulfil an essential function in the control of stomatal density and distribution. sdd1 loss of function mutations result in two- to fourfold increase of stomatal density and the formation of clustered stomata (i.e., stomata that are not separated by intervening pavement cells). This gene is predominantly expressed in meristemoids / guard cell mother cells and the encoded protein appears to accumulate at the extracelular surface associated to the plasma membrane. SDD1 is therefore proposed to be involved in the creation of an extracelular signal emanating from meristemoids / guard cell mother cells that controls the development of cell lineages forming stomatal complexes. Further characterised members of this Arabidopsis gene family may be involved in lateral root formation and cuticle development. To gain insight into the roles of Arabidopsis subtilase genes, they will be subjected to a functional genomics program including sequence analysis, expression studies, and mutant identification and characterisation. Putative or confirmed T-DNA insertion mutations have hitherto been identified for 41 genes.

W27 Functional analysis of plant nucleobase and vitamin C transporters

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Nucleobases play central and indispensable roles in the growth and development of higher plants, such as DNA and RNA metabolism. In addition, they can be used as nitrogen sources e.g. for the biosynthesis of secondary compounds such as cytokinins, some alkaloids or of ureides in legumes. Ascorbate (vitamin C) represents one of the main antioxidants in plants protecting plant cells against oxidative stress. Ascorbic acid occurs in all cell compartments and in the apoplast, with concentrations ranging from 0.6 mM in vacuoles up to more than 30 mM in chloroplasts and the citosol. Thus, distribution of ascorbic acid throughout the cell requires extensive transport across most, if not all, cellular membranes. Interestingly, both structurally unrelated compounds, nucleobases and ascorbate, are transported by the same family of transport proteins, the Nucleobase-Ascorbate Transporters (NATs). The *Arabidopsis thaliana* genome contains at least 12 genes encoding putative NAT proteins with yet unknown functions. The aim of this project is to elucidate the transport specificities and the physiological roles of the various members of the NAT protein family in organelles and the plasma membrane during development and response to environmental (stress) conditions, e.g. by heterologous expression of the proteins in yeast cells and the ascomycete *Aspergillus nidulans*, using Northern blot assays, cDNA microarrays, by (sub)cellular localisation studies, and by isolation and characterization of insertion mutants at the molecular, biochemical and physiological levels.

W28 *APS*, an Arabidopsis SBP-box gene affecting sporogenesis

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SBP-box genes share a highly conserved DNA-binding domain, the SBP-domain, and likely act as plant specific transcription factors. In Arabidopsis, SBP-box genes comprise a structurally heterogeneous family of 16 members known as *SPL* genes but little is known about their role in development. Screening large transposon-mutagenised populations of Arabidopsis plants allowed the isolation of mutant alleles for one of the Arabidopsis *SPL* genes. In homozygous condition, the mutants alleles all exhibited a strong reduction in fertility. Due to its most obvious mutant phenotypic effect, the corresponding *SPL* gene has been renamed to *APS* for *ABERRANT POLLEN SAC*. *APS* represents the first SBP-box gene in Arabidopsis of known function in development.

W29 Cation diffusion facilitator (CDF) proteins of Arabidopsis- A role in metal homeostasis?

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Essential metal cations like those of Cu, Fe, Mn, Ni or Zn are required as micronutrients. Since these transition metal cations display high affinities for organic ligands, and since some of them are able to participate in single-electron transfer redox reactions, availability inside the plant has to be tightly controlled in a metal homeostasis network involving uptake, sequestration, movement, trafficking and storage of these metals. In a functional genomics approach we are addressing the role in metal homeostasis of 13 Arabidopsis membrane proteins with some homology to *Saccharomyces cerevisiae* Zrc1p, *Ralstonia eutropha* CzcD, mammalian ZnTs and AtZAT of the cation diffusion facilitator family (Van der Zaal et al., Plant Physiol. 119, 1047-1055, 1999).
To functionally characterize these proteins, heterologous expression was performed in a zinc-hypersensitive mutant of *S. cerevisiae*. Analysis of transcript levels by real-time PCR revealed differential expression in plant organs. In roots and shoots transcript levels responded to altered supply of transition metals. Transient expression of GFP fusion proteins is in progress in order to determine the subcellular localization of the proteins *in planta*. A number of T-DNA knockout lines have been obtained.

W30 Functional analysis of SCF ubiquitin ligase complexes

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Skp1, Cdc53 (Cullins) and F-box proteins are the major components of so-called SCF complexes that function as ubiquitin ligases. SCF complexes are involved in the regulation of the proteasome-dependent degradation of a multitude of intermediates from diverse signalling pathways in yeast, animals and plants. The sequencing of the complete Arabidopsis genome revealed a large number of SCF sub-units including 6 Cullin, 19 ASK and more than 400 F-box proteins. Until now, it is unknown which of these components will assemble into complexes and which domains are involved in this process. During our research program, we will analyze the function of F-box proteins that carry protein domains similar to the C-terminus of EID1. EID was characterised as an important component of phytochrome A-dependent light regulation in Arabidopsis. We want to examine the composition of SCF complexes related to EID1 and its homologous proteins. Putative target proteins of these F-box proteins should be identified. Furthermore, we want to elucidate the peptide structures that are necessary for the assembly of ASK proteins and the extremely variable F-box domains. To analyze these interactions, we perform domain swab experiments using the C-terminal domain of EID1 together with different F-box domains of other proteins involved in Arabidopsis signal transduction. We also plant to establish *in vitro* competition assays using matrix-bound ASK proteins and labelled F-box proteins.

W31 Functional analysis of a calcium sensor protein/serine-threonine kinase signaling network in Arabidopsis

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Calcium signals in plant cells are elicited by a variety of stimuli such as hormones, light and stress factors. Perception and transmission of these signals often involve the specific action of calcium sensor proteins. We have recently described a new family of calcineurin B-like (CBL) calcium sensor proteins from Arabidopsis and identified a group of plant-specific serine-threonine protein kinases (CIPKs, CBL-interacting protein kinases) as targets of these sensor proteins. CBL-CIPK interaction is mediated by the NAF domain, a specific protein-protein interaction module defining this group of calcium regulated kinases. Several of these kinases have been implicated in various signalling pathways mediating responses to stress, hormones and environmental cues. Whole genome database analyses and subsequent cDNA cloning revealed that the CBL-CIPK signalling network comprises of 10 calcium sensor proteins and 25 interacting kinases.
This project aims to comprehensively explore the function of these signalling components by a combination of expression analyses, yeast protein-protein interaction studies and reverse genetic approaches. Here I will summarize the current status of the project with special emphasis to the interaction studies and mutant analyses. In addition, I will discuss results, which might have implications for the genetic analysis of other complex gene families.

W32 The Arabidopsis network of heat stress transcription factors

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Heat stress transcription factors (Hsfs) are the terminal components of a signal transduction chain mediating the activation of genes responsive to both heat stress and a large number of chemical stressors. The complexity of the plant stress response systems correlates with an unusual multiplicity of regulatory proteins. Sequencing of the *Arabidopsis thaliana* genome revealed the existence of 21 ORFs encoding putative Hsfs. By structural characteristics and phylogenetic comparison, the 21 representatives are assigned to three classes and 14 groups. The sequencing of the *Arabidopsis thaliana* genome allows now a more detailed discussion on the plant Hsf system based on the combination of sequence comparison and results from the fairly advanced functional analysis of tomato Hsfs. The project aims at the comparative analysis of these Hsfs with respect to their expression patterns, activator functions, intracellular localization and above all their interaction with other Hsfs (heterooligomerization) or other regulatory proteins (coregulators). A central aspect will be the characterization of the complex promoter architecture of the *Arabidopsis thaliana* Heat stress protein (Hsp) genes and the role of Hsf combinations for efficient activation of these genes (synergism between Hsfs). The results will serve as a basis for more advanced investigations of the Hsf network and its cross talks with the general stress signalling pathways of plants.

W33 Network of regulatory interactions of MADS-box proteins during floral organ development in Arabidopsis

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In Arabidopsis, the MADS-box family of transcription factors comprises more than 100 members. The majority of the Arabidopsis MADS-box genes is not yet characterized, neither structurally nor functionally. The best known and most intensively studied are the floral homeotic MADS-box genes *AGAMOUS (AG), APETALA3 (AP3), PISTILLATA (PI)* and *APETALA1 (AP1),* which control development and the establishment of organ identity of the four floral organs. The importance of these MADS proteins for floral organogenesis is manifested in the dramatic phenotypic alterations of organs, when the respective genes are inactivated by mutations. Other members of this protein family are involved in initiation of flowering, root development and tissue differentiation. The basic structure of the MADS proteins allows, besides their binding to DNA, to undergo multiple protein-protein interactions that seem to be the molecular basis of complex regulatory networks.
Goal of the project:

- The fully sequenced genome allows quick in silico identification/isolation of new MADS-box genes.
- In order to uncover the function of these genes, knock-out mutants will be isolated by reverse genetic approaches.
- To gain insight in the complex network of interactions of MADS proteins, we want to apply yeast-based techniques to isolate interaction partners. Yeast two-hybrid screens will be conducted with MADS-box proteins to isolate their interaction partners. This allows, in a next step, to carry out large scale ternary factor trap screens in a search for ternary/quaternary protein interactions.

W34 Functional dissection of the WRKY family of transcription factors involved in senescence and pathogen defense

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The plant-specific family of WRKY transcription factors most likely comprises 73 functional members in *Arabidopsis thaliana*. Although still poorly studied, WRKY proteins appear to play an important regulatory role under stress-related conditions, particularly during defense against phytopathogenic microbes, but are also involved in certain developmental processes such as senescence and trichome development. WRKY factors bind to cis-regulatory elements designated as W boxes (C/TTGACC/T) and can function both as transcriptional activators and as repressors. Aim of this project is to use defined Arabidopsis WRKY "knockout" and inducible WRKY overexpressor lines for comparative protein profiling studies with wild type plants under several defined stress-related conditions including senescence. Peptides differentially appearing in such profiles will be identified by MALDI-MS/MALDI-TOF. In addition, we hope to identify WRKY interacting partners with the help of epitope-tagged WRKY proteins.

W35 Functional analysis of the heptahelical transmembrane MLO protein family in Arabidopsis

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Barley MLO is the prototype of the only sequence-diversified family of heptahelical transmembrane (7-TM) proteins in plants and is the sole family member whose function is known so far. The *Mlo* wild type gene modulates plant defence and may have specifically evolved to ensure a balance between mutually inhibitory defence responses to different pathogen species. The existence of *Mlo* homologues in moss and their absence in red and green algae indicates that the origin of the gene family coincides with the early stages of land plant evolution. The Arabidopsis genome encodes 15 family members and each of the genes is expressed. The common 7-TM scaffold topology of MLO family members, the location in the plasma membrane, and the within-species sequence diversification are characteristics reminiscent of heptahelical transmembrane receptors in fungi and animals. We have shown that MLO mediates defence modulation via direct Ca2+-dependent interaction with calmodulin and independently of heterotrimeric G-proteins. MLO-mediated defence modulation likely involves small GTP-binding proteins of the ROP family.
Our project aims to identify the function(s) of the 15 MLO family members in Arabidopsis. Independent mutant screens failed to identify an Arabidopsis mutant that resembles the barley *mlo* phenotype, suggesting either functional diversification between monocot and dicot *Mlo* homologues or genetic redundancy of Arabidopsis family members. By means of systematic knock out mutagenesis of Arabidopsis *Mlo* genes, we will directly examine whether one or several family members function in plant defence modulation. We will also explore whether family members or subfamilies are engaged in other biological processes.

W36 Receptor-like kinases implicated in the activation of plant disease resistance

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Leucine-rich repeat receptor-like kinases (LRR-RLK) have been implicated in the activation of plant disease resistance. However, in neither case have these proteins been shown to directly interact with avirulence gene-encoded proteins or pathogen surface-derived elicitors. Since genes encoding components of signalling pathways in eukaryotes are often found to be transcriptionally up-regulated by the corresponding stimulus, we exploit this to identify LRR-RLK and LRRP-encoding genes potentially involved in disease resistance. To analyze and isolate signal perception complexes mediating the activation of plant defense responses we have infected Arabidopsis thaliana Col-0 plants with avirulent viral, bacterial, oomycete, and fungal pathogens as well as with elicitors of plant defense reactions. RNA from infected plants was used in microarray assays using the 8.3 k gene chip from Affymetrix. Eight out of 59 LRR-RLK genes present on the chip were found to be activated by specific treatments while transcript levels of another subset of genes decreased. Induced genes will be characterized with respect to their function in plant defense signalling (gene inactivation by T-DNA insertion or RNA interference and subsequent analysis of the mutants in infection assays). Preliminary results of this work will be discussed.

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