1 Genetic analysis of auxin signaling: A long road to a short pathway

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The plant hormone auxin has been studied since Charles and Francis Darwin published "The Power of Movement in Plants" in 1880. Nearly 30 years of genetic experiments in Arabidopsis recently culminated in the description of the auxin-signaling pathway. Auxin acts by promoting the degradation of transcriptional regulators called Aux/IAA proteins through the action of the ubiquitin protein ligase SCF^{TIR1}. In recent work, TIR1, the F-box protein subunit of SCF^{TIR1}, was shown to function as an auxin receptor. Auxin binds directly to TIR1 to promote the interaction between TIR1 and the Aux/IAA proteins. However, loss of TIR1 has a modest effect on auxin response indicating that additional auxin receptors must be present. Indeed genetic and biochemical studies indicate that three additional F-box proteins called AFB1, 2 and 3 also regulate auxin response. Like TIR1, these proteins interact with the Aux/IAA proteins in an auxin dependent manner. The successive loss of TIR1 and the AFB proteins causes a progressively more severe phenotype. Plants that are deficient in all four proteins are auxin insensitive and exhibit a severe embryonic phenotype similar to the mp/arf5 and bdl/iaa12 mutants. These results indicate that TIR1 and the AFB proteins define a new family of auxin receptors that collectively mediate auxin responses throughout plant development. Remarkably, auxin acts directly on the ubiquitination machinery to promote the very rapid degradation of transcriptional repressors.

2 Bioinformatics

Chris Town
TIGR

Introductory Remarks

3 Improving Protein Functional Classification Through Structural Phylogenomics

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Protein families evolve a multiplicity of functions and structures through gene duplication, domain shuffling, speciation and other processes. As numerous studies have shown, annotation transfer by homology is associated with systematic errors on these data. Phylogenomic analysis, combining phylogenetic tree construction, integration of experimental data, and differentiation of orthologs and paralogs, has been shown to address these errors and improve the accuracy of functional classification. The explicit integration of structure prediction and analysis in this framework, which we call structural phylogenomics, enables biologists to visualize the correlation between changes in protein structure and function in protein superfamily evolution, and also improves the accuracy of functional annotation by phylogenomic inference.

To facilitate the use of this approach by the greater scientific community, the Berkeley Phylogenomics Group is developing a Universal Proteome Phylogenomic Explorer with open access to all investigators in the public and private sectors. As of March 25, 2006, our library includes almost 10K "books" representing protein structural domains and whole-chain protein families and over 700K HMMs for individual protein families and subfamilies. This resource enables the identification of either a structural domain or a match to a whole-chain protein family for over 75% of the genes in the Arabidopsis genome, as well as similar coverage for other eukaryotic genomes. Each family book contains a multiple sequence alignment, one or more phylogenetic trees, subfamilies identified using the SCI-PHY algorithm, hidden Markov models for the family and predicted subfamilies, predicted 3D structure(s), matches to PFAM domains, GO annotations and evidence codes, predicted transmembrane domains and signal peptides, predicted active sites and other functional residues, and hyperlinks to relevant literature. Users can browse the library, use keyword search to retrieve families associated with particular biological processes, or submit sequences for classification to families or subfamilies. We currently focus primarily on protein structural domains, proteins encoded in animal genomes and proteins involved in plant disease resistance, but plan to eventually represent all protein families found in all branches of the Tree of Life.

4 Transcriptional coordination of the metabolic network in Arabidopsis thaliana

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Patterns of co-expression can reveal networks of functionally-related genes and provide deeper understanding of processes requiring multiple gene products. We performed an analysis of co-expression networks for 1,330 genes from the AraCyc database of metabolic pathways in Arabidopsis. We found that genes associated with the same metabolic pathway are, on average, more highly co-expressed than genes from different pathways. Positively co-expressed genes within the same pathway tend to cluster close together in the pathway structure, while negatively correlated genes typically occupy more distant positions. The distribution of co-expression links per gene is highly skewed, with a small but significant number of genes having numerous co-expression partners but most having fewer than ten. Genes with multiple connections (network hubs) tend to be single-copy genes, while genes with multiple paralogs are co-expressed with fewer genes, on average, than single-copy genes, suggesting that the network expands through gene duplication, followed by weakening of co-expression links involving duplicate nodes. Using a network-analysis algorithm based on co-expression with multiple pathway members (pathway-level co-expression), we identified and prioritized novel candidate pathway members, regulators, and cross-pathway transcriptional control points for over 140 metabolic pathways. To facilitate exploration and analysis of the results, we provide a Web site (http://www.transvar.org/at_coexpress/analysis/web) listing analyzed pathways with links to regression and pathway-level co-expression results. These methods and results will aid in the prioritization of candidates for genetic analysis of metabolism in plants and contribute to the improvement of functional annotation of the Arabidopsis genome.

5 New MapMan Dimensions

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The MapMan data visualisation software has been developed for the visualization of large datasets, such as transcript, metabolite and/or protein profiling experiments. The package visualises this data in the context of pre-existing biological knowledge such as biological pathways or regulative processes. These pathways can either be self-made or chosen from the rich selection of pathways that come preinstalled with MapMan (nearly 40 diagrams, covering areas such as primary metabolism, hormone metabolism, receptor kinases etc.). Thus, it is possible to get an idea of the biology behind one's experiments by simply loading the experiment into MapMan.

Even though, MapMan has originally been designed to represent only one kind of multi-parallel data at the same time, here we represent the adaptation of MapMan to multi platform multi level assays, by using different glyphs, as well as the introduction of statistics which gives a quick overview of the pathways that might be affected the most.

Furthermore, due to the extent of multi-condition multi-chip experiments which are currently conducted by more and more researches, we developed a new tool building upon the MapMan framework to visualize these datasets.

This new tool relies upon a statistics engine to summarize a chip experiment using the functional MapMan ontology. The resulting summary is then displayed graphically and can be adapted interactively before final export. Thus, it enables the researcher to investigate into several chip experiments at once with an immediate insight into the changes in biological processes underlying the experiments. Further in depth analysis of individual chips can then be done using the classical MapMan tool by choosing maps which are automatically suggested.

6 The Arabidopsis Information Resource (TAIR)

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TAIR (http://arabidopsis.org) provides a comprehensive Arabidopsis data resource, integrating genes, proteins, maps, clones, seed and DNA stocks, polymorphisms, germplasms, genetic markers, microarray experiments, biochemical pathways, community members and published literature. **Genome Annotation:** TAIR's first genome release, TAIR6 (Nov 2005) contains 26,751 protein coding genes, 3818 pseudogenes and 838 non-coding RNA genes. Changes include 437 new genes, 9 genes removed, updates to 973 genes including 831 updates to coding sequences, 14 gene splits, 7 gene merges and the addition of 1200 new splice variants, bringing the total number of genes with splice variants to 3159 (10%). No changes were made to the chromosome sequences for this release. Access to the fully annotated chromosome sequences in TIGR xml format as well as fasta files of cDNA, cds, genomic and protein sequences and lists of added, deleted and changed genes are available at: ftp://ftp.arabidopsis.org/home/tair/Genes/TAIR6_genome_release. The release is also available from NCBI. Our next release is planned for fall 2006. **Gene Function:** TAIR annotates gene function and expression through manual literature curation using Gene Ontology (GO) and Plant Ontology (PO) controlled vocabulary terms. We have recently switched from using the TAIR anatomy and development terms to the PO structure and growth/developmental stage terms for annotating expression patterns. In early 2005 we shifted our literature curation focus to current papers, resulting in more rapid integration of new information into TAIR. We have begun an effort to curate phenotype data using a combination of free text and PO terms. Genes and pathways in our AraCyc biochemical pathways database have been significantly updated and a new function (download genes) added to the Pathway page.

Website improvements: We have added "Download All" functions to Gene and Microarray Expression. External links on locus pages have been expanded to include several microarray data sources. A new navigation structure including dropdown menus, a navigation bar and more intuitive portal pages is in the final stages of development. Data from TAIR can be viewed on detail pages, downloaded from search results pages or our bulk downloads page (http://arabidopsis.org/tools/bulk/) or downloaded from our ftp site. The community is encouraged to submit data on gene structure and function, expression patterns, phenotypes, markers, protocols, gene families and seed and DNA stocks. Instructions and forms for submitting data and materials can be found at http://arabidopsis.org/info/data_submission.jsp. A TAIR beginners' workshop and an advanced workshop on data submission will be presented.

7 Web services for Arabidopsis data integration

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Exploiting the full potential of Arabidopsis thaliana (functional) genomic data is currently hindered by the limited mechanisms of availability and the lack of integration. Web services provide a mechanism whereby data residing at many different locations can be seamlessly integrated to provide the user with richer data sets. A demonstration project is initiated with the encouragement of the Multinational Arabidopsis Steering Committee that oversees and attempts to integrate functional genomics efforts. The aim is to successfully deploy web services at eight research sites in Europe and eight in the US that vary both in the kinds of data that they host, and in the level of expertise at the sites. The aim is to improve availability and integration of Arabidopsis data through a network of standardized web service providers. Based on the BioMoby project, service development is relatively easy, relying on code generators for service skeletons. A growing number of web services are available and can be combined into custom workflows. Some example applications will be demonstrated, as well as query and visualization tools utilizing the web services.
http://bioinfo.mpiz-koeln.mpg.de/araws

8 A systems biology approach to understanding root development

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Most eukaryotic development begins with a fertilized egg, which undergoes a series of cell divisions to generate a multicellular organism in which the diverse cell types function in harmony. Central processes in development include creating distinctions between cells and producing coordination among different cells so that they function as units. In plants, both processes have been shown to rely heavily on cell-to-cell communication and activation and/or repression of subsets of genes. While signaling and transcription are equally important for development, high through-put techniques for identifying the nodes and links in transcriptional networks have matured more rapidly. For plants the simplifying aspects of development in an organ such as the root, make it highly tractable for the application of these approaches. The Arabidopsis root develops continuously from four sets of stem cells in its tip. These stem cells divide asymmetrically to regenerate themselves and produce a daughter cell, which in turn divides asymmetrically to generate the first cells of each of the root lineages. Because plant cells don't move, these cell lineages are constrained in cell files. Thus, in the root, each stage of development is found in a specific set of cells along the longitudinal axis, with the youngest cells in each file being closest to the initials. The other simplifying aspect of root development is that, at least for the four outer layers of cells, the root can be viewed as a radially symmetric cylinder.

To identify the transcriptional networks that regulate plant development three datasets are needed: 1) global expression profiles; 2) cellular localization of transcription factors; and 3) transcription factor targets. To understand the role of transcriptional networks in development, each of these datasets needs to be at cell-type specific resolution. Methods for acquiring these types of data will be discussed and results from many of the cell types in the root will be presented. Preliminary results on the effects of perturbing the networks with environmental stimuli will also be described.

9 Systems Biology and Reverse Engineering of Metabolic Pathways Using Sparse GGM

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The analysis of genetic regulatory networks was greatly advanced by the availability of large data sets from high-throughput technologies such as DNA microarrays. The genome-wide, parallel monitoring of gene activity will increase our understanding of the molecular basis of pathway functions and their cellular network context. In simple eukaryotes or prokaryotes, gene expression data has been combined with two-hybrid data and phenotypic data to successfully predict protein-protein interactions and transcriptional regulation on a large scale. In higher organisms, however, little is known about regulatory control mechanisms and pathway networks on a larger scale. As a first step we have focused on isoprenoid metabolism, which is universally conserved and essential for cell survival. Arabidopsis has to independent pathways that function in the cytoplasm and chloroplast (www.pnas.org/cgi/content/abstract/100/11/6866. We developed a novel graphical Gaussian modelling (GGM) approach to elucidate the regulatory network of the two isoprenoid biosynthesis pathways bases on large scale expression data (http://genomebiology.com/2004/5/11/r92/abstract). When applying this approach to infer a gene network, we detect modules of closely connected genes and candidate genes for cross-talk between the isoprenoid pathways. Genes of downstream pathways also fit well into the network. We evaluated our approach in a simulation study and using the yeast galactose utilization network. Connected genes were independently validated using Genevestigator? (www.genevestigator.ethz.ch), a novel powerful software suite for visualization of microarray and other data in their biological context.

10 Functional Analysis of Regulatory Flexibility in the Circadian Clock: Transcriptomic and Reporter Gene Assays of Circadian Phase

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Circadian clocks provide organisms with the ability to temporally coordinate their biological and physiological activities with the predictable changes in the daily environmental cycle. Studies in Arabidopsis and Cyanobacteria indicate that correct synchronisation of the clock with the external environmental cycle provides advantages to an organism's fitness. The selective benefits the clock provides are brought about in part by the targeting of biological events to the correct phase of the daily cycle. The core of the plant clock was proposed to be a transcription translation feedback loop involving the genes *TOC1*, *CCA1* and *LHY*. Mathematical modelling of this basal clock in our lab has demonstrated that it is insufficient to explain experimental data and has led to the extension of the network to include multiple, interconnected feedback loops. Differing responses of the clock controlled genes *CAT3* and *CAB* to light and temperature signals in a previous study suggests the potential to de-couple the output from selected loops (1). Unlike a single-loop clock, multiple interconnected loops with different light inputs allow the clock to track multiple events in the day/night cycle, for example both dusk and dawn. The temporal relationship between such events is dynamic throughout the year. Thus, the ability to track more than one event provides the clock with greater flexibility to target the plants biological activity to optimal phases, maximising its selective advantage. Using a combination of micro-array and *LUCIFERASE* reporter gene data we look at the effect of differing environmental conditions on the mechanisms and output from the clock within a small network of core clock genes and on an extended network of several thousand genes controlled by clock. New methods for scoring circadian phase and clustering rhythmic timeseries will be presented. The implications of the observed flexibility in regulation for the clock network structure will be discussed.

(1) Michael, TP et al., 2003 PNAS 100(11)

11 LATCA: a Library of Biologically Active Small Molecules for Plant Chemical Genomics.

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University of Toronto, University of Toronto, University of Toronto, University of Toronto, University of Toronto

To facilitate the use of chemical genomics for dissecting problems in plant biology we have assembled a collection of ~2000 compounds that can be obtained in screening-ready format from the Cutler laboratory. We have named this collection LATCA, for **L**ibrary of **A**c**T**ive **C**ompounds on **A**rabidopsis. The library is biased towards compounds defined as biological activity using an etiolated hypocotyl growth inhibition assay performed with three commercial libraries: Chembridge Diverset (10K), Sigma's LOPAC (1.2K) and Microsource's Spectrum (2K).

Our screens of these three libraries identified ~1100 compounds as active on the Col-0 ecotype. These compounds and an additional 900 analogs and novel structures were purchased in milligram quantities and assembled to make LATCA. Approximately 300 of the LATCA compounds are known to be active in plants (i.e. plant growth regulators and herbicides), yeast (fungicides), mammals (FDA approved compounds) or bacteria (antibiotics). The remaining compounds are novel drug-like structures and natural products with uncharacterized target sites and modes of action.

The phenotypes induced by LATCA compounds were analyzed at a single dose in etiolated seedlings, documented with publication quality images and classified by noting the presence or absence of 26 phenotypes relating to the apical hook, hypocotyl, root and cellular morphology. These observations enable phenotype-based clustering which reveals major phenotypic classes such as auxin mimics and isoxaben mimics. The compounds have also been clustered according by structure and comparison of the two datasets has uncovered known and novel core structures that produce similar phenotypes.

To demonstrate the utility of the LATCA for new screens, we used it in a microscope-based screen of an ER-targeted GFP marker for disruptors of ER morphology. This yielded a new compound that we have named Eroonazole that causes ER tubules to loose integrity and form balloon like structures; the effects of Eroonazole suggest that it may affect a component(s) required for maintenance of cortical ER-tubule integrity. A re-screen of ~3000 known biologically active agents failed to identify any other compounds which induce a similar ER-tubule phenotype. Thus, the LATCA can be used to identify new classes of biological active molecules in plants and should be a useful resource for labs interested in both identifying new leads and scanning known biologically active molecules for activity in a process of interest.

12 Design and testing of an Arabidopsis small RNA microarray

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Approximately 2,500 small RNA's were selected from the Arabidopsis small RNA MPSS database (Lu et. al., 2005) for addition to the Agilent Arabidopsis whole genome array. These included all known Arabidopsis miRNA's from the Sanger miRNA database, miRNA candidates, and a selection of siRNA's. The initial design was based on Barad et al (2004). The 21-25 nt small RNA's were printed on the array "tethered" by a 35-39 nt sequence found not to cross hybridize to any eukaryotic genome. The small RNA's were printed in both sense and antisense orientations (about 5000 features total) to allow both for flexibility in labeling and to have a negative control for miRNA's and duplicate probes for siRNA's. Total RNA from Arabidopsis root, inflorescence and total seedlings was isolated and PEG precipitated to enrich for low molecular weight (>100nt) RNA's. 1-2ug of the low molecular weight RNA fraction was directly labeled, and the arrays hybridized and scanned as described by Babak et al (2004). The results obtained were compared to the existing Arabidopsis small RNA MPSS database and to existing knowledge of Arabidopsis miRNA expression to validate the array design. The arrays were then stripped as described by Hu et al (2004) and rehybridized to the corresponding cRNAs to obtain the equivalent gene expression data. Further experiments planned are to compare small RNA designs where the "tether" linking the 21-25nt small RNA will be either 20-24nt (total 45mer) or a concatenated small RNA (45 or 60mer). The ultimate goal will be to design a microarray containing the more than 10, 0000 known small RNAs to be available to the worldwide Arabidopsis community.

13 Phenotypic Functions of Bathochromic Mutant Phytochromes A Conferring Shade Tolerance to *Arabidopsis thaliana*

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Phytochromes are the phyto-photoreceptors mediating a variety of photomorphogenic responses to red/far-red wavelength lights. Phytochromes exist in two photochromically interconvertible isomers, the red-light absorbing Pr and far-red light absorbing Pfr forms. The ratio of red and far-red wavelength lights (R:FR ratio) determines the photoequilibrium of the Pr and Pfr forms to induce and/or modulate plant's photoresponses. Thus, a high R:FR ratio induces photoequilibrium of phytochrome toward the functionally active form, Pfr. In contrast, a low R:FR ratio under shade conditions shifts the photoequilibrium to the inactive Pr form, resulting in shade avoidance responses as exhibited by multiple phenotypic changes including stem elongation, internodes extension, retardation of leaf growth, and early flowering. In crop plants, these shade-avoiding responses lead to significant losses of yields. In the present study, we have developed bathochromic mutant phytochromes whose Pr-absorption spectra move to longer wavelength so that they can be activated even at low R: FR ratios to suppress shade avoidance responses in plants. We have also characterized their *in vivo* functions in terms of shade tolerance. Changing highly conserved amino acid residues in the bilin lyase domain of *Avena* PhyA, we obtained several absorption wavelength-shifted mutants including bathochromic mutants, for examples, F389A with a 6 nm shift, F307R/C371A with an 8 nm shift, as well as a hypsochromic mutant whose the Pr absorption spectrum moves to shorter wavelength such as R317E with an 8 nm shift. For the *in vivo* functional analysis, several mutant phytochrome genes were introduced into *phyA*-deficient *Arabidopsis* and shade tolerance of their transgenic plants was examined in both seedlings and adult plants under different shaded conditions. The transgenic plans with bathochromic phytochromes suppressed hypocotyl elongation in seedlings under shaded conditions, and exhibited leaf morphologies indicative of shade tolerance in adult plants. Furthermore, early flowering induced by the shade was retarded. On the other hand, the transgenic plants with a hypsochromic mutant displayed an increased sensitivity to shade. Our results show that the bathochromic mutant phytochromes confer shade tolerance to plants.

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14 Regulation of Phosphate Starvation Responses in Arabidopsis. Transcriptional Control and Beyond

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Phosphorous is an essential nutrient for all organisms. Despite large fluctuations in external phosphate (Pi), the preferential form in which P is absorbed, control of Pi homeostasis within the plant is a critical determinant for proper growth performance. In these organisms, a highly elaborated and complex regulatory system is involved in the control of Pi homeostasis. In this system, the crucial role of a MYB transcription factor, PHR1, has been established. Transcriptome analysis has revealed that PHR1 binding motifs (P1BS) are over-respresented in promoters of Pi induced genes, but not in Pi repressed genes, despite their repression is reduced in the phr1 mutant. Therefore, PHR1 primaryly acts as a transcription activator, and its repressor effect is indirect. Reporter gene analyses have shown that a multimerised P1BS motif is sufficient to promote Pi starvation responsiveness from an artificial promoter. However, in the context of natural promoters, additional cis-regulatory motifs are needed to respond to Pi starvation. This is in line with phylogenetic footprinting data that reveals conservation of several motifs in the promoters of this type of genes. Additional regulatory components of Pi homeostasis, beyond transcriptional control of Pi starvation responses, have also been identified by others and us. Among others, these include Pi starvation induced miRNAs and other non-protein coding RNAs which control interorgan Pi distribution, and PHF1, a Pi starvation induced protein that is required for proper trafficking of Pi transporters from the endoplasmic reticulum to the plasmamembrane.

15 Autophagy is Required for Plants to Survive under Abiotic Stresses

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Autophagy is a process in which cytoplasmic components are degraded in the vacuole to provide raw materials and energy for the maintenance of essential cellular functions. It occurs when organisms are subjected to environmental stress conditions or during certain stages of development. Upon induction of autophagy, a portion of cytoplasm is surrounded by a double membrane structure to form an autophagosome. The outer membrane of the autophagosome then fuses with the vacuole and the inner membrane and its contents are degraded by vacuolar hydrolases. Using two autophagy markers, monodansylcadaverine (MDC) and GFP-AtATG8e, we found that abiotic stresses such as nutrient deprivation, high salinity, osmotic stress and oxidative stress can induce autophagy in Arabidopsis plants. Autophagy-defective RNAi-*AtATG18a* transgenic plants are more sensitive to these abiotic stresses than wild type plants, implying an important role for autophagy in the response to these conditions. Under oxidative stress, RNAi-AtATG18a plants accumulate a higher level of oxidized proteins than wild type plants, due to a lower degradation rate. Furthermore, when treated with concanamycin A to inhibit vacuolar enzyme activity, oxidized proteins can be detected in the vacuole of wild type root cells but not RNAi-*AtATG18a* roots cells. Together, our data suggests that autophagy is involved in degrading oxidized proteins during oxidative stress in Arabidopsis. The physiological role of autophagy in other abiotic stresses is under investigation.

16 The Molecular Basis of Temperature Compensation in the Arabidopsis Circadian Clock

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Plants, in common with most eukaryotes have evolved an endogenous 24 hour clock. Both light and temperature provide important cues to entrain the clock to local time. This allows the organism to accurately predict and prepare for the onset of dawn and dusk. The entrained clock is utilised to orchestrate metabolism, gene expression and development. Collectively, this regulation increases growth, photosynthesis and ultimately the survival of plants. For the circadian clock to be useful to the plant it must maintain robust and accurate rhythmicity over a broad range of physiological temperatures, buffering against seasonal fluctuations in temperature. This temperature buffering or temperature compensation is a key feature of all circadian systems.

In Arabidopsis, ambient temperature affects the rhythmic accumulation of transcripts encoding the clock components TIMING OF CAB EXPRESSION 1 (TOC1), GIGANTEA (GI) and LATE ELONGATED HYPOCOTYL (LHY). The amplitude and peak levels increase for the TOC1 and GI RNA rhythms as the temperature rises, whereas they decrease for the LHY RNA rhythm. A dynamic balance between GI and LHY appears to be essential for temperature compensation at temperature with the clocks in both lhy and gi mutants having a reduced ability to buffer against temperature. While at lower temperature the role of LHY is replaced by CCA1. GI is also required for the maintenance of robust and accurate rhythms of CAB gene expression and leaf movement at high and low temperatures, whereas at 17°C, GI is apparently dispensable for free-running circadian rhythms. Such a function is analogous to that of the two splice variants of the Neurospora clock protein FREQUENCY (FRQ).

This new insight into how the clock maintains robustness and accuracy at different temperatures is likely to have implications for enhancing the performance of plants. It is feasible that this could be used to extend the geographical range of a crops and allow the development of new varieties able to cope better with global climate change.

17 ELF4 Point Mutations Affect Phase and Period Properties of the Arabidopsis Circadian Clock

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EARLY FLOWERING 4 (*ELF4*) functions in the *Arabidopsis thaliana* circadian clock. To further study the role of *ELF4* in relation to the properties of the circadian oscillator, we have performed a series of molecular and genetic experiments. Our expression analysis of *ELF4* lends support to the current model of the circadian clock. *ELF4* peaks at subjective night, in agreement with *ELF4* regulation of *CCA1*, a core-clock gene. This observation can also explain the attenuated *CCA1* expression in the *elf4* loss-of-function mutant. We found a similar loss of *LHY*, another morning gene of the oscillator, in the *elf4* mutant. Accordingly, we see that the *CCA1* level is elevated in *ELF4* over-expression (*ELF4*-ox) plants. However, *ELF4* targets *CCA1* rather than *LHY*, because *LHY* levels are not increased in *ELF4*-ox. The expression of the evening clock gene *TOC1* is elevated in the *elf4* mutant and reduced in *ELF4*-ox, which supports *ELF4* action on *CCA1* in the *CCA1*/*LHY*-*TOC1* loop. To further characterize *ELF4*, we have taken a reverse-genetic approach using TILLING. In addition, we isolated *ELF4*-like sequences from several plant species and we were able to predict that the new *elf4* missense alleles would have subtle mutant phenotypes, because many conserved residues were not affected by TILLING mutagenesis. *Promoter:luciferase* reporters are integrated in the new *elf4* lines, and clock outputs are also measured by monitoring cotyledon movement rhythms. Our preliminary findings include new elf4 phenotypes. Short period, early phase, and late phase alleles are evident in the TILLING collection. The strongest alleles have reduced *CCA1:LUC* expression and approach arrhythmicity under free-run. Our current model is that the central region of ELF4 is critical for proper function. We are currently extending this genetic analysis to be able to link the ELF4 protein to other clock components.

18 Signaling Network of Plant Immunity

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Induction of systemic acquired resistance (SAR) involves salicylic acid (SA)-mediated activation of pathogenesis-related (PR) genes. Genetic analysis showed that this process involves regulation of both the positive regulator NPR1 and the negative regulator SNI1. The molecular functions of these two regulators have been studied. In the absence of the SA signal, NPR1 protein is present in an oligomeric form through intermolecular disulfide bonds. Reduction of NPR1 to monomer occurs after SAR induction as a result of SA-induced redox changes in the cell. Only the monomeric form of NPR1 is translocated to the nucleus. Mutants that affect the intermolecular disulfide bond formation showed constitutive accumulation of NPR1 monomer in the nucleus and constitutive NPR1 activity. Conversely, inhibition of NPR1 monomer formation prevents the onset of SAR. The stability of the NPR1 monomer depends on SA-mediated phosphorylation of the protein. In the nucleus, NPR1 interacts with TGA transcription factors to activate PR gene expression. In addition to NPR1, SNI1 has been found to be an important repressor of PR genes and SAR. A saturating mutagenesis was performed to understand how SNI1 inhibits PR gene expression and how SAR induction causes inactivation of SNI1. Through these studies, we have defined regions in SNI1 that are important for its repressor activity and regions that are involved in SAR induction. In addition, one of sni1 suppressors, ssn1, has been cloned and characterized. Using microarray technology, the direct transcriptional target genes for NPR1 have been identified and their functions in conferring SAR resistance revealed.

19 Controls over oxylipin biogenesis

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Lipid oxidation reactions play central roles in the wound response and in pathogenesis and numerous genetic studies have confirmed the importance of the canonical jasmonate pathway in plant defense. A central question is how is oxylipin biogenesis initiated and correctly controlled? Initiation of oxylipin biogenesis is a necessary first step in signaling. We have taken several genetic approaches designed to gain insights into how cells control the production of two classes of lipid oxidation products. Jasmonate synthesis is initiated by lipoxygenases (LOXs) and a versatile genetic screen was designed to detect altered LOX activity in Arabidopsis. The ongoing screen yielded fou (fatty acid oxygenation upregulated) mutants displaying an increased capacity to catalyse the synthesis of LOX metabolites. In fou2, transcript levels for AtLOX2 and 3 are between 2- and 3-fold higher in mature leaves of the mutant. Quantitative oxylipin analysis identified increased jasmonate levels in both healthy and wounded leaves, and the plants displayed strongly enhanced resistance to the fungus Botrytis cinerea. Unlike many jasmonate signaling mutants, fou2 did not display altered sensitivity to jasmonic acid. Higher than wild-type LOX activity, short petioles, anthocyanin accumulation and enhanced resistance in fou2 depend fully on a functional jasmonate response pathway. fou2 carries an amino acid substitution in a voltage gated ion channel and should yield insights into how enzymatic controls over jasmonate synthesis are activated and exerted in response to insult. In very severe stress, such as hypersensitive responses and in some abiotic stresses, uncontrolled lipid oxidation can begin to occur. The oxidation and fragmentation of polyunsaturated fatty acids that occurs in these conditions generates oxylipins with poorly characterized roles. Experimental evidence now reveals that some of these compounds, and in particular reactive electrophile species (RES), may influence gene expression and play roles in cell damage and cell survival. The conditions necessary for the initiation of the formation of some of these compounds are now becoming clearer as are their potential roles.

20 The MOS4-Associated Complex is an Important Regulatory Node in NPR1-Independent Innate Immunity Signaling

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Plant disease resistance is the consequence of an innate defense mechanism mediated by Resistance (R) proteins. The dominant mutant *snc1* constitutively expresses *Pathogenesis-related* (*PR*) genes and exhibits enhanced resistance to both the virulent bacterial pathogen *Pseudomonas syringae maculicola* (*Psm*) ES4326 and virulent oomycete pathogen *Peronospora parasitica* (*Pp*) Noco2. *snc1* accumulates high levels of endogenous salicylic acid (SA), and has a dilapidated morphology. *SNC1* encodes an RPP5 homolog – a single amino acid change in the region between the NB-ARC and LRR of SNC1 renders this R-protein constitutively active. To identify signaling components downstream of *snc1*, we employed a genetic screen to search for suppressors of *snc1*.

One mutant isolated in this screen, *modifier of snc1, 4* (*mos4*), almost completely suppressed *snc1* morphology, constitutive *PR* gene expression, SA accumulation and resistance to virulent pathogens. The *mos4* single mutant exhibited enhanced disease susceptibility (EDS) to *Psm* ES4326 and *Pp* Noco2. *mos4* was also more susceptible to avirulent pathogens, the resistance to which represents both PAD4- and NDR1-dependent signaling pathways. Several lines of evidence showed that MOS4 mediated resistance was independent of NPR1 in *snc1*-mediated and basal defense. *MOS4*, identified by map-based cloning, encodes a small protein with predicted protein-protein interaction domains. Subcellular localization of MOS4-GFP shows that MOS4 is localized to the nucleus.

To illuminate the biochemical function of MOS4, a yeast-2-hybrid screen was conducted. A trancription factor, MOS4-Associated Complex Protein 1 (MAC1), was shown to interact directly with MOS4. The *mac1* insertion mutant displayed the phenotypes of EDS to virulent and avirulent pathogens similar to *mos4*. In addition, *mac1* also partially suppressed *snc1* morphology and enhanced resistance. Both MOS4 and MAC1 have homologs in humans and fission yeast that interact directly and have been implicated in several different biological processes. We believe that MOS4 and MAC1 are part of a novel multi-protein complex that represents an essential regulatory node in NPR1-independent resistance signaling.

21 bZIP10-LSD1 Antagonism Modulates Basal Defense and Cell Death in *Arabidopsis* Following Infection

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Plants use sophisticated strategies to balance their responses to oxidative stress. Programmed cell death, including the hypersensitive response (HR) associated with successful pathogen recognition, is one outcome regulated by reactive oxygen in various cellular contexts. The Arabidopsis basic leucine zipper (bZIP) transcription factor AtbZIP10 binds consensus G- and C-box DNA sequences. AtbZIP10 shuttles between the nucleus and the cytoplasm, likely via interaction with the export receptor AtXPO1. Surprisingly, AtbZIP10 can be retained outside the nucleus by LSD1, a protein that protects Arabidopsis cells from death in the face of various oxidative stress signals. We demonstrate that AtbZIP10 is a positive mediator of the uncontrolled cell death observed in *lsd1* mutants. *AtbZIP10* and *LSD1* act antagonistically in both pathogen-induced HR and basal defense responses. LSD1 likely functions as a cellular hub, where its interaction with AtbZIP10 and additional, as yet unidentified, proteins contributes significantly to plant oxidative stress responses.

22 Identification of *Arabidopsis* Ortholog(s) of a Regulator of Systemic Acquired Resistance in Tobacco

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Systemic Acquired Resistance (SAR) is induced upon infection of plants with an incompatible pathogen. A signal for SAR is sent from the site of infection through the phloem to uninfected healthy tissues of the plant to induce resistance. Salicylic Acid Binding Protein 2 (SABP2) of tobacco converts methyl salicylate (MeSA) to SA and is required for the establishment of SAR in tobacco (Forouhar et al., 2005, Proc. Natl. Acad. Sci. USA 102: 1773-8; Kumar and Klessig, 2003, Proc. Natl. Acad. Sci. USA 100: 16101-6). Our working model is that MeSA might constitute, at least in part, the SAR signal sent from the primary infected leaf to systemic uninfected tissues of plants where it is converted by SABP2 to the active defense compound SA. In this study, we sought to identify the functional homolog(s) of SABP2 in Arabidopsis, which encodes 18 SABP2-Like (AtSB2L1-18) proteins. We found that three recombinant AtSB2L proteins (AtSB2L1, 7, and 9) of eleven tested displayed esterase activity in vitro with a preference for MeSA as a substrate. SA binding could be detected for AtSB2L9, and to a much lesser extent for AtSB2L7, but not for AtSB2L1. A T-DNA insertion knock out (KO) mutant of AtSB2L9 was SAR-defective in 4 out of 5 experiments. Moreover, inducible expression of AtSB2L9 in SABP2-silenced tobacco complemented the SAR-deficient phenotype of the silenced plants. From these results, we conclude that AtSB2L9 is a functional homolog of SABP2 and that the role of MeSA in SAR appears to be conserved between different plant genera. Interestingly, an AtSB2L1 KO mutant exhibited a stronger SAR response than wild type plants, while the double AtSB2L1 X AtSB2L9 KO mutant developed normal SAR. Further experiments are currently underway and a model for the role of AtSB2L1 and AtSB2L9 in SAR in Arabidopsis will be discussed.

23 Histone methyltransferases that control DNA methylation

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In Arabidopsis, heterochromatin formation is guided by double-stranded RNA (dsRNA), which triggers methylation of histone H3 at lysine 9 (H3 mK9) and CG plus non-CG methylation on identical DNA sequences. At heterochromatin targets including transposons and centromere repeats, H3 mK9 mediated by the SUVH4/KYP histone methyltransferase (MTase) is required for maintenance of non-CG methylation by the CMT3 DNA MTase. Although SUVH4 is the major H3 K9 MTase, the related SUVH5 and SUVH6 proteins also have histone MTase activity in vitro and contribute to maintenance of H3 mK9 and CMT3-mediated non-CG methylation in vivo. Strikingly, the relative contributions of SUVH4, SUVH5, and SUVH6 to non-CG methylation are locus-specific. For example, SUVH4 and SUVH5 together control transposon sequences with only a minor contribution from SUVH6, whereas SUVH4 and SUVH6 together control a transcribed inverted repeat source of dsRNA with only a minor contribution from SUVH5. This locus-specific variation suggests different mechanisms for recruiting or activating SUVH enzymes at different heterochromatic sequences. A triple suvh4 suvh5 suvh6 mutant loses both mono- and di-methyl H3 K9 at target loci. The suvh4 suvh5 suvh6 mutant also displays a loss of non-CG methylation similar to a cmt3 mutant, indicating that SUVH4, SUVH5, and SUVH6 together control CMT3 activity.

24 Elucidating the Small RNA Component of the Transcriptome

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Small RNAs such as miRNAs and siRNAs are a powerful regulatory force in most eukaryotes because they can function to shut off genes at multiple levels. Deep sequencing of the small RNA component of the transcriptome is an important step toward elucidating the impact of small RNAs on individual genes and the genome as a whole. In collaboration with Solexa, Inc. we have developed a method based on massively parallel signature sequencing ("MPSS") and used it to identify more than 75,000 different small RNA sequences from Arabidopsis [Lu et al., Science 309:1567-1569, 2005; http://mpss.udel.edu/at. Additional progress on Arabidopsis small RNA analysis will be described including experiments with microarrays containing about 2500 small RNA sequences. By applying MPSS sequencing to the small RNAs of rice, we have found that the small RNA profile of this organism is far more complex. Nearly 150,000 different sequences were found in a small RNA library made from rice flowers. Further analysis of the rice small RNA sequences will be discussed with emphasis on the features that differ between Arabidopsis and rice, or are common to these plants. Funded by the NSF, DOE and the USDA.

25 Chaperone Hsp90 As A Molecular Mechanism Of Genetic And Environmental Canalization

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We have shown that manipulation of Hsp90 results in the expression of altered phenotypes, which are partially due to uncovering normally hidden genetic variation. Exposure of such "buffered" genetic polymorphisms may also be accomplished by environmental alteration. Hence, if such polymorphisms are widespread, natural selection may be more effective at producing phenotypic change in suboptimal environments. The frequency and identity of buffered polymorphisms in natural populations have yet to be assessed.

We performed a pilot study using pharmacological inhibition of Hsp90 to assess the potential of quantitative genetics to identify Hsp90 buffered polymorphisms. Specifically, we undertook QTL analysis of an A. thaliana developmental response, hypocotyl elongation in the dark. Our study identified two novel QTLs which contribute to hypocotyl length only upon Hsp90 inhibition. Fine mapping of one QTL revealed that multiple loci responded to a decrease in Hsp90 function, suggesting that such loci may be frequent. Analysis of hypocotyl elongation across 60 divergent accessions yielded association data supporting our linkage analysis. It also revealed previously unknown correlations to both geographical factors such as latitude and environmental factors such as temperature variance. Integration of association and linkage data suggests candidate causal polymorphisms, which we are currently verifying.

For a broader analysis of the frequency of Hsp90 buffered polymorphisms, we have created a set of 200 recombinant inbred lines that are stably reduced in Hsp90 via RNAi targeting, along with a corresponding control set. We used a novel array-based method to rapidly genotype these lines at over 200 markers. These lines have been analyzed for a range of life-history traits. Comparison of QTL maps from the Hsp90-reduced and control sets demonstrates that Hsp90 manipulation reveals the effects of cryptic polymorphisms in many different traits. Some traits are affected by multiple revealed loci.

Thus, phenotypic changes revealed by Hsp90 manipulation have a multigenic basis. Many different polymorphisms are buffered by Hsp90, and Hsp90 buffering affects a wide trait spectrum. Combined with the previously observed effects of altered environments on Hsp90-dependent phenotypes, our results support the proposition that the raw potential for evolutionary change is highly dependent on the genetic and environmental context, with Hsp90 in a central position linking the environment to the translation of genotype to phenotype.

26 BONSAI: Loss-of-function Epigenetic Mutation Induced in the ddm1 (decrease in DNA methylation) Background

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A DNA hypomethylation mutation ddm1 induces various types of developmental abnormalities through heritable changes in other loci. One of such ddm1-induced abnormalities, which is characterized by aberrant phyllotaxy and dwarf phenotype, was named bonsai (bns). The bns phenotype was heritable even in the presence of wild type DDM1 copy, but the inheritance was unstable and the expressivity was variable, suggesting that the bns phenotype may be due to epigenetic alteration rather than genetic mutation. We performed linkage analysis and identified a gene that is specifically silenced in the bns line. No change in DNA sequence was identified, but bisulfite-sequencing analysis revealed that the repression of BNS transcription is associated with DNA hyper-methylation in whole BNS region. In addition, we found production of small RNA from bns epi-allele, which was not found in parental lines. Notably, BNS gene has an insertion of transposable element in 3'UTR region, implicating that changes in epigenetic state of the transposon affect BNS gene expression. Possible mechanism of this paradoxical phenomenon, local DNA hypermethylation in the background of global hypomethylation, will be discussed in the context of RNA-directed DNA methylation.

27 Measuring Selection on Natural Variation

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Brown University

Plants must integrate information from several environmental signalling pathways in order to flower at the appropriate time under dynamic real-world conditions. Both the environmental signals and the ecological factors that exert selection on the resulting phenotypes vary in space and time, so the optimal flowering response may vary geographically or across seasons. Arabidopsis thaliana is an ideal model system for investigating the functional and evolutionary significance of natural variation in the converging signalling pathways regulating reproductive timing. Ecotypes of A. thaliana from diverse climates exhibit considerable natural variation in developmental timing, which in turn is associated with variation in fitness under field conditions. Geographic patterns of genetic variation in life history traits suggest adaptation to climate, and the climate of origin of European ecotypes predicts colonization success in New England . Natural variation at the major flowering time gene FRIGIDA is associated with natural variation in developmental timing, as well as fitness, under field conditions. However, patterns of natural selection depend upon genetic background and differ across geographic sites and seasonal environments. The opportunity now exists to understand the adaptive evolution of flowering time from molecular polymorphism to ecological mechanisms.

28 Evolutionary Mechanisms of Light Response Adaptation

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We are using novel genomics tools and techniques to understand the evolutionary mechanisms of light response adaptation. Whole genome tiling array allow for detailed studies of within species variation at the genetic, epigenetic, transcriptomic level. I will present new results detecting structural variation, alternative splicing, methylation profiling, and allele specific expression that depend on wild genotypes, season like environments, and on interactions between them. These methods are revealing the genetic basis of adaptation and the signaling pathways involved.
http://naturalvariation.org
http://naturalsystems.org

29 Common alleles of PHYTOCHROME C mediate natural variation in flowering and growth responses of *Arabidopsis thaliana*

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Light plays a major role in several aspects of plant development including seedling growth and floral transition. Wild strains of Arabidopsis thaliana show extensive phenotypic variation in both these responses. Quantitative trait locus (QTL) mapping studies and subsequent molecular genetic analysis have identified several loci that contribute natural variation in flowering and light responses. However, most of the identified natural alleles are specific to strains and/or phenotypes. Here we report that common alleles of the photoreceptor gene PHYC confer natural phenotypic variation in both flowering time as well as seedling growth across wild strains of A. thaliana. We show that the short day early flowering phenotype of the Fr-2 strain is due to a naturally occurring loss-of-function allele of PHYC. In addition, quantitative complementation suggest that the commonly used laboratory strain Ler carries a weak allele of PHYC. Sequence analysis, haplotype tagging, phenotypic association and a comparison of available QTL maps reveal two functionally distinct haplogroups of PHYC that could account for flowering time and light sensitivity QTLs detected across several RIL populations. The PHYC haplogroups display a FRIGIDA-dependent latitudinal cline in their distribution that is stronger than what is reported for FLOWERING LOCUS C. A genomic scan with 67 SNPs with matching allele frequency with of that of PHYC across 165 eurasian strains reveals an excess of significant p-values indicating population structure. Nevertheless, PHYC is ranked the highest among the 67 SNPs for association with latitude indicating the PHYC haplogroups are under diversifying selection in A.thaliana. Our analysis together with previous findings suggests the photoreceptor genes to be major agents for local adaptation in A.thaliana.

S.B and S.S contributed equally to this work

30 Recent Selection in the Arabidopsis Genome: FRIGIDA and Beyond

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The widespread geographical distribution and abundance of phenotypic diversity in natural Arabidopsis isolates suggest that adaptations to local conditions may be common. Recent developments in methods to detect the signature of such adaptation at the DNA level, as well as the emergence of genome-wide polymorphism data from Arabidopsis, may lead to the identification of loci involved in adaptation. However, the standard neutral model is likely not an appropriate null model for most samples of Arabidopsis.

We formulated a novel statistic for testing recent selection that attempts to correct for population structure present in species-wide samples. We tested whether common deletion alleles at the FRIGIDA (FRI) locus with a strong effect on flowering time without vernalization show evidence of recent selection in a species-wide sample of 96 individuals. We determined at least one allele to be significant by comparing the FRI alleles to the genome-wide distribution of the test statistic estimated from approximately 1,100 short DNA fragments, avoiding the unrealistic assumptions of the standard neutral model. Based on patterns of linkage disequilibrium, selection appears to have occurred during the last several thousand years. The increase and spread of these alleles could represent selection for weediness in response to the spread of agriculture. We also identified several other regions of the genome as candidates for harboring alleles involved in local adaptation.

31 Independent ancient polyploidy events in the sister families Brassicaceae and Cleomaceae

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Recent studies have elucidated the ancient polyploid history of the *Arabidopsis thaliana* (Brassicaceae) genome. The studies concur that there was at least one polyploidy event occurring some 14.5 to 86 million years ago, possibly near the divergence of the Brassicaceae from its sister family, Cleomaceae. Using a comparative genomics approach, we asked whether this polyploidy event was unique to members of the Brassicaceae, shared with the Cleomaceae, or an independent polyploidy event in each lineage. We isolated and sequenced three genomic regions from diploid *Cleome spinosa* (Cleomaceae) that are each homoeologous to a duplicated region shared between At3 and At5, centered on the paralogs of *SEPALLATA* and *CONSTANS*. Phylogenetic reconstructions and analysis of synonymous substitution rates support the hypothesis that a genomic triplication in *Cleome* occurred independently of and more recently than the duplication event in the Brassicaceae. There is a strong correlation in the copy number (single versus duplicate) of individual genes, suggesting functionally consistent influences operating on gene copy number in these two independently evolving lineages. However, the amount of gene loss in *Cleome* is greater than in *Arabidopsis*. The genome of *C. spinosa* is only 1.9x the size of *A. thaliana*, enabling comparative genome analysis of separate but related polyploidy events.

32 Flowering and Vernalization

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The timing of the floral transition has significant consequences for the reproductive success of plants. Plants need to gauge when both environmental and endogenous cues are optimal before undergoing the switch to reproductive development. To achieve this, a complex regulatory network has evolved consisting of multiple pathways that quantitatively and antagonistically regulate the genes whose activity causes the transition of the meristem to reproductive development.

The Dean group has focused on a set of pathways that regulate the strong floral repressor, FLC. Vernalization, the acceleration of flowering by prolonged cold, epigenetically down-regulates FLC and antagonizes the function of FRIGIDA, which up-regulates FLC. Genes of the autonomous pathway such as FCA and FY function in parallel to vernalization to repress FLC expression. The talk will address how these pathways interact to regulate FLC expression at different stages in the plant life-cycle and how these have changed in natural Arabidopsis variants adapted to very different growth conditions.

33 Seven Cells in the Ovule: Functional Analysis of the Female Gametophyte Transcriptome

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Plants have evolved a life strategy with alternating generations, continuous postembryonic development and the absence of a distinct germ line. These specialized features have important implications for the development of gametes and seeds. The establishment of the gametophytic (haploid) generation represents an evolutionary driving force that might be at the origin of genetic and epigenetic mechanisms necessary to ensure that seed development is tightly regulated. Strikingly, many flowering species have developed strategies to form embryos from somatic cells or without previous fusion of sperm and egg. Our group investigates the genetic basis and molecular mechanisms that regulate female gametogenesis and early seed formation. We have used cell-directed RNA interference (RNAi) to systematically inactivate genes acting in the developing female gametophyte, showing that specific chromatin remodeling factors are essential for haploid nuclear proliferation prior to cellularization. An in-depth transcriptional analysis of the Arabidopsis female gametophyte by Massively Parallel Signature Sequencing (MPSS) indicates that unique small non-coding RNAs (sncRNA) and microRNA-processing enzymes are active in the fully differentiated ovule, suggesting that the epigenetic control of early seed formation depends on distinct and specific mechanisms that prevail during female gametogenesis. We believe that this type of regulation is crucial to understand the developmental events that distinguish sexual from asexual reproduction in flowering plants.

34 The molecular identification of RDO2 and RDO4 reveals new aspects of the seed dormancy mechanism

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Seed dormancy is defined as the failure of a viable seed to germinate under favorable conditions. Besides having an adaptive role in nature by optimizing germination to the best suitable time, a tight control of dormancy is important in crop plants. Extensive physiological studies have identified the involvement of different factors, including hormones, but the molecular mechanism underlying this process is still unknown. Our lab aims to identify the molecular pathways that lead to establishment and release of dormancy in Arabidopsis.

One of our strategies is to study mutants with altered dormancy levels. Four mutants with reduced dormancy (rdo) and mild pleiotropic phenotypes have been identified in mutagenesis screens (Léon-Kloosterziel et al. 1996; Peeters et al. 2002). We recently cloned two of these (rdo2 and rdo4), using a map based approach. RDO2 encodes a transcription elongation factor and RDO4 a RING finger protein with homology to an evolutionarily conserved yeast protein that is required in vivo for both H2B ubiquitination and H3K4 methylation. This points to a role of these general mechanisms in dormancy.

We are also interested in the chromatin structure of dormant and non-dormant seed and found first indications that the very low level of activity in the nucleus of dormant seed is reflected in its chromatin organization. At present, we study the role of RDO4 in this process.

35 Temporally and Spatially Regulated Auxin Biosynthesis Controls the Formation of Floral Organs and Vascular Tissues

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The molecular mechanisms of auxin biosynthesis in plants have not been well understood, although auxin has been implicated in almost every aspect of plant growth and development. Here we show that the YUC family of flavin monooxygenases in Arabidopsis plays an essential role in auxin biosynthesis and plant development. Overexpression of each YUC gene in Arabidopsis leads to auxin overproduction, but the single loss-of-function mutants of the YUC genes have no obvious developmental defects. Certain combinations of double, triple, and quadruple yuc mutants display severe defects in the formation of floral organs and vascular tissues. The developmental defects of yuc mutants are rescued by tissue specific expression of the bacterial auxin biosynthesis gene iaaM, but not by exogenous auxin. The YUC genes are not ubiquitously expressed, rather their expressions are mainly limited to meristems, young primordia, and vascular tissues. Inactivation of YUC genes leads to specific reduction of DR5-GUS in cells where the YUC genes are expressed, without affecting the GUS-staining of other tissues. These results demonstrate that the YUC genes are key auxin biosynthesis components and that spatially and temporally regulated auxin synthesis by the YUC flavin monoxygenases is essential for the formation of floral organs and vascular tissues.

36 A RAV gene negatively regulates FT expression and extremely delays flowering

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The transition to flowering is a developmental process that should be tightly regulated in order to guarantee the reproductive success of the plant. The moment at which this transition occurs is determined by both endogenous and environmental signals. In Arabidopsis there are four major floral promotion pathways that converge at the transcriptional regulation of the genes called floral pathway integrators: FT, SOC1 and LFY. These integrators are responsible of the floral meristem identity (FMI) gene activation.

FT is a potent promoter of flowering in response to photoperiod and vernalization. It is mainly expressed in the vascular tissue and it is the major primary target of CO in leaves. Several recent reports have helped to understand how a gene that is overall expressed in leaves, is able to induce flowering in the shoot apex. The suggested model considers FT mRNA as at least part of the floral stimulus that travels through the phloem to the meristem to trigger the transition to flowering. Once in the meristem, FT is able to interact with FD, and together induce the expression of the FMI genes. However, it has also been suggested that this system probably involves a complex network of inductive and repressive activities that should act together to strictly control the flowering time.

We have identified a novel regulator of FT expression that belongs to the RAV family. RAV proteins are transcription factors that contain both an AP2 and a B3 DNA binding domain. Mutations in this RAV gene cause early flowering whereas its overexpression extremely delays flowering by reducing the levels of FT. Moreover, 35S::RAV in lfy mutant background mirrors the double ft lfy mutant phenotype with all flower structures converted into leaves. In addition, we have seen that the RAV protein binds the 5'UTR of FT in vitro, supporting the idea of being a direct negative regulator of FT expression and therefore of the transition to flowering.

37 Promoting Stomatal Development

Dominique Bergmann
Stanford University

Stomatal development is a simple model in which to study the integration of lineage, local cell contacts and the environment during cell fate decisions and the creation of organized tissues. Stomata are structures in the epidermis of aerial organs that function as conduits for the exchange of carbon dioxide (CO_2) and water vapor between the plant and the atmosphere. Stomatal development is characterized by an orchestrated series of asymmetric cell divisions followed by a single symmetric division. Asymmetric divisions contribute to the overall number of cells and their arrangement in the epidermis, whereas the final symmetric division is responsible for the creation and differentiation of stomatal guard cells. Recent studies have highlighted the roles of cell-cell signaling mediated by LRR-containing receptors and a MAP kinase cascade in repressing the inappropriate acquisition of stomatal identity. We sought to identify genes required to promote stomatal development. Using a variety of experimental approaches including genetic screens in sensitized backgrounds, transcriptional profiling of plants that lack or overproduce stomata [1] and protein interaction tests, we identified a set of related transcription factors that appear to be major controllers, in turn, of each step in the stomatal development pathway--from the initial choice to undergo an asymmetric division to enter the stomatal lineage through the final differentiation into guard cells. Several of these genes appear to act as switches between continued division and terminal differentiation. We will present extensive characterization of FAMA, a gene required for the final step in the pathway, but will also highlight the roles of its earlier-acting relatives and the functional relationships among these genes.

[1] Bergmann, D.C., Lukowitz, W., and Somerville, C.R. (2004). Stomatal development and pattern controlled by a MAPKK kinase. Science 304, 1494-1497.

38 Root Patterning and Cell Polarity in Arabidopsis Roots

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Recent data suggest that transport-dependent auxin maxima are important for development not only in the root but also during embryogenesis and in shoot-derived organs. It now becomes an important question how auxin as a patterning cue induces specific downstream pathways to mediate diverse effects. The Arabidopsis PLETHORA1 and PLETHORA2 genes encode transcription factors required for stem cell specification and can ectopically induce root identity. PLT expression is auxin-inducible, depends on auxin response factors and follows auxin accumulation patterns during embryogenesis and in post-embryonic root development. PLT genes translate auxin accumulation into region- and cell type specification patterns, and interact with the SHORTROOT-SCARECROW pathway that plays a role in patterning the root stem cells. Mutations in new PLT genes reveal that the PLT gene clade extensively regulates expression of the PIN facilitators of polar auxin transport in the root and this contributes to a specific auxin transport route that maintains stem cells at the appropriate position. We are currently investigating the role of graded PLT expression in this control.

To establish what determines specific cellular activities downstream of these patterning events, we analyzed the FEZ and SOMBRERO genes that are required specifically for root cap stem cell action. We found that they encode plant-specific putative transcription factors whose expression is fine-tuned by mutual regulation. Interestingly, these genes control the division plane of epidermal/lateral root cap stem cells in opposite ways.

39 FOUR LIPS/MYB124 and MYB88 enhance PIN transcription

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The shape and position of plant organs are often determined by local auxin accumulation. Extensive physiological and genetic studies have revealed a key role for PIN auxin efflux facilitators in establishing sites with high auxin content crucial for developmental changes. However little is known on the mechanisms regulating PIN expression during organogenesis. Here, we show that a pair of related Arabidopsis MYB transcription factors (FLP/MYB124 and MYB88) act redundantly in enhancing PIN expression during lateral root development. Upon auxin treatment, flp myb88 double mutant roots form a multilayered pericycle and broad, disorganized lateral root primordia , a response reminiscent of mutants defective in polar auxin transport. Double mutants also display a strong reduction in PIN auxin inducibility, suggesting that FLP and MYB88 act upstream of PIN activation. Furthermore, ChIP analysis using FOUR LIPS/MYB88 antibodies show a direct interaction of FLP/MYB88 to a specific region in the PIN promoter. Taken together these data reveal for the first time a direct interaction between a transcription factor and a PIN promoter.

40 Examination of the Mechanisms of SHORT-ROOT Cell-to-Cell Signaling

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Cell-to-cell communication is essential to coordinate developmental events in both animals and plants. Patterning of organs and specification of cell types requires intercellular signaling to communicate positional information. The signaling molecules involved in these developmental events include small molecules, polypeptide ligands, and small RNAs. Surprisingly, in plants, transcription factors can also traffic from cell to cell and serve as signaling molecules. One such transcription factor, SHORT-ROOT (SHR), moves between cells in the Arabidopsis root where it is responsible for the asymmetric division of the ground tissue and differentiation of the endodermis. We have have identified multiple regions of the SHR protein that are required for movement and have found an unexpected correlation between the ability of SHR to localize to the nucleus and its ability to move. In addition, we have isolated proteins that interact with SHR whose identities suggest a role for the plant endomembrane system in intercellular protein movement.

41 DORNROESCHEN (DRN) and DRN-LIKE Redundantly Control Cotyledon Initiation and Meristem Development in Arabidopsis thaliana

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Embryo development and the establishment of a functional SAM and subsequent organ formation are amongst the most fundamental processes of plant development. DRN and DRN-LIKE are two linked gene paralogues encoding AP2 domain transcription factors of the ERF (Ethylene Response Factor) type, expressed from early embryo stages. Insertion mutants in either DRN or DRN-LIKE genes show cotyledon and embryo cell division defects and a double mutant between drn and a weak allele of drn-like reveal an additional more extreme phenotype where the embryo basal domain is absent, demonstrating that both genes act redundantly to control embryo patterning. This functional redundancy is further revealed when drn is combined with a stronger allele of drn-like gives rise to plants lacking cotyledons. pin-like embryos produce an active SAM and initiate leaves directly. However, the primary growth axis arrests in pin-like and radialised structures and subsequent development continues by secondary meristems and axillary inflorescences. This phenotype demonstrates both genes play a fundamental role in meristem and organ formation and additionally show that cotyledons are not necessary for SAM or leaf formation. A yeast two hybrid screen revealed that both DRN and DRN-LIKE interact with members of the classIII HD-ZIP protein family, comprising PHAVOLUTA, PHABULOSA, CORONA, REVOLUTA and ATHB8, via a novel C-terminal domain and the AP2 domain of DRN and DRN-LIKE. To place DRN and DRN-LIKE functions in pathways involving genes whose loss-of-function give rise to similar cotyledon defects, such as the CUC, PINOID and PIN genes, a genetic approach has been taken. Double mutant combinations between drn and drn-like and the cuc1, 2 or 3 mutants reveal individual contributions of DRN and DRN-LIKE to different CUC gene pathways.

42 Where are all the proteins? Sub-cellular compartmentation of the Arabidopsis proteome as a key foundation for post-genomic analysis of metabolism

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A core issue in understanding the function of the proteins of predicted and unknown function encoded in the Arabidopsis genome is an understanding of the location and associations of these proteins at a sub-cellular level. Transcriptome data have played a central role in defining the set of genes that are expressed and likely form protein products in different tissues, during development and in response to environmental signals. However, on the issue of sub-cellular location of the protein products, transcripts alone are unable to play an experimental role. Sub-cellular location is a central aspect of cellular metabolism. It allows compartmentation of function and the operation of similar enzymes in different pathways and even in opposite directions within the same cell. Several routes are open to provide a foundation of subcellular location for the 10,000s of non-redundant proteins expected to be synthesized in Arabidopsis. Sub-cellular fractionation and proteomic analysis by mass spectrometry is leading the way in defining this set in a systematic but undirected fashion, clone-base proteomics using GFP:target protein fusions provides a directed route to follow a pre-defined set of proteins, while bioinformatics targeting prediction programmes and sequence and sequence motif comparisons to other eukaryotic models can provide a wide-scale set but this potentially has a high false-positive rate. Future developments to complete this process and provide data on most Arabidopsis proteins will require advances in the purification of organelles, reduction in the impact of the visualization tags on protein localization of tag:target constructs and the development of new tools for prediction of protein location. Further, detailed knowledge of protein-protein interactions in their sub-cellular locations and affinity based purification of subsets of low abundant proteins from sub-cellular compartments and sub-compartments will be required to provide a fuller assessment of protein composition and protein interactions in Arabidopsis. With reference to mitochondria, the current data and recent directions in the study of this organelle's proteome will be presented as a typical model for studies of sub-cellular proteome analysis in Arabidopsis that are helping to re-shape our understanding of metabolism.

43 The Visible Plant Cell: Biosensors And Bioreporters In vivo physiological imaging using fluorescent indicator proteins

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It has recently become possible to directly image metabolic processes in living cells, with good spatial and temporal resolution (Journal of Biological Chemistry 2003, 278:19127; Proceedings of the National Academy of Sciences of the USA 2002, 99: 9846). The technique takes advantage of changes in Forster resonance energy transfer (FRET) between donor-acceptor pairs of fluorescent proteins genetically fused to a ligand-binding domain (recognition element). The technique is general, requiring only a ligand-dependent conformational change in the recognition element to transduce binding into a macroscopic fluorescence observable, and has been demonstrated with a variety of protein-ligand pairs. The greatest wealth of functional recognition elements has been found in the superfamily of periplasmic binding proteins (so called ?antibodies for small molecules?), although a number of other scaffold topologies are being explored. The nanosensors have been extensively optimized by a variety of rational, computational and empirical protein engineering techniques, both to optimize signal, to increase sensor robustness, to modulate ligand-binding affinity and change ligand-binding specificity, and to create spectrally-orthogonal nanosensors for simultaneous multiplex monitoring. The sensors have been deployed in vitro in biological solutions such as beer, and in vivo in living animal, yeast, and plant cells. The sensors comprise analytes such as sucrose, maltose, glucose, phosphate and amino acids have been developed and are used to quantify subcellular analyte levels in mutant collections to systematically identify factors that control ion and metabolite hoemostasis.

Fehr M., Takanaga H., Ehrhardt D.W. & Frommer W.B. (2005) Evidence for high-capacity bidirectional glucose transport across the endoplasmic reticulum membrane by genetically encoded fluorescence resonance energy transfer nanosensors. Mol. Cell Biol. 25, 11102-11112. Lalonde, S., Looger L.L. & Frommer W.B. (2005) Shining light on signaling and metabolic networks by genetically encoded biosensors. Curr. Opin. Plant Sci. 8, 1-8. Deuschle K., Okumoto S., Fehr M., Looger L.L., Kozhukh, L. & Frommer W.B. (2005) Construction and optimization of a family of genetically encoded metabolite sensors by semi-rational protein engineering. Protein Sci. 14, 2304-2314.

44 Peroxisomal ATP Import Is Involved In Fatty Acid Oxidation

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Storage lipid mobilization is critical for seed germination. Until the photosynthetic apparatus is established, the seedling degrades fatty acids released from lipid to fulfil its carbon and energy requirements. The subsequent break down of fatty acids occurs in the peroxisome by the b-oxidation. To enter this pathway, the free fatty acids have to be activated to their respective Coenzyme A derivatives by ATP dependent Acyl-CoA sythethases. Fulda et al. (2004) identified two peroxisomal enzymes which are involved in the fatty acid activation. A loss-of-function led to seedlings with an inhibition of the b-oxidation.

The inability to synthesize ATP necessitates ATP import into peroxisomes. Therefore, a specific transport protein is required to mediate the import of ATP. Here we present the analysis of three candidates for peroxisomal ATP transporters in *Arabidopsis thaliana*. We have demonstrated that two of these proteins are located in the peroxisomal membrane in yeast and *in planta* and that they complement a yeast mutant impaired in peroxisomal ATP import. In addition, we have studied the biochemical properties of recombinant proteins using a proteoliposome system which revealed that they catalyze an ATP/AMP exchange.

Arabidopsis T-DNA insertion lines and RNAi plants were generated to analyze the function of peroxisomal ATP import for fatty acid oxidation. Because the b-oxidation is also involved in phytohormone biosynthesis, like jasmonic acid, a detailed screen to test the impact of impaired ATP uptake into peroxisomes will be presented.

45 Functional Identification of Arabidopsis Flavonoid 7-*O*-rhamnosyltransferase Gene by Co-expression Analysis

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It is well known that genes involved in a certain biosynthetic pathway are expressed coordinately. Conversely, clusters of co-expressed genes are functionally related in many cases. By co-expression analysis which utilizes correlation coefficients based on 771 GeneChip data from AtGenExpress, we attempted to identify the function of unknown glycosyltransferase genes related in flavonoid metabolism.

In Arabidopsis, flavonoids are highly modified by glycosylation. The flavonoid structures imply that at least eight glycosyltransferases (GT) are involved in this pathway and only four GT genes were identified. GTs are encoded by 107 genes in Arabidopsis. Their primary structures are not sufficient to estimate their functions because of their higher diversity.

By coexpression analysis of public database, ATTEDII, we found a GT gene, *UGT1*, which is highly correlated with flavonoid biosynthetic genes. The deduced amino acid sequence is similar to that of flavonol 7-*O*-glucosyltransferase. The T-DNA insertion mutants didn't contain flavonol 7-*O*-rhamnosides. GST-fused recombinant UGT1 protein can convert kaempferol 3-*O*-glucoside to kaempferol 3-*O*-glucoside 7-*O*-rhamnoside. These results show that *UGT1* encodes flavonoid 7- *O*-rhamnosyltransferase (At7RhaT). Real time PCR analysis showed that transcripts of *At7RhaT* are accumulated abundantly in buds. It's consistent with the flavonoid accumulation pattern in Arabidopsis organs.

46 Functional and Informatic Analyses of the Arabidopsis Plastid

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Michigan State University

The plastid has a large but manageable number of proteins (< 4,500) that are targeted to or synthesized within it. This makes it an excellent target for a functional genomics in Arabidopsis. To understand the metabolic functioning of the plastid, it is necessary to connect individual genes with functions via direct morphological and biochemical assays as well as informatic analyses. We are using three approaches to functionally annotate these genes. Our central approach is parallel phenotypic screening of knockout mutants. This includes plant, seed and chloroplast morphology, seed C/N ratio, chlorophyll fluorescence kinetics as well as analysis of a diverse set of metabolites that are synthesized in the plastid (lipids, amino acids, tocopherols, chlorophylls and starch). The second approach is to use contextual information from bacteria to assign gene function in plants (http://www.figresearch.com/). The third approach is homology-based protein structural modeling for the inferred gene products. We will perform metabolic flux analysis on selected mutant lines to study how gene products act dynamically to determine the physiology of plant cells.

We have configured multiple phenotypic assays and have developed an internal website for seed stocks and data entries. The results of a large feasibility study and progress of the project will be presented. The results of these laboratory and informatic analyses will also be available at the project website (www.plastid.msu.edu).

47 Suppression of Light Signaling in Darkness

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The four-member SPA protein family of Arabidopsis functions in concert with the E3 ubiquitin ligase COP1 to suppress photomorphogenesis in dark-grown seedlings. Here, we demonstrate that SPA proteins are, moreover, essential for photoperiodic flowering. Mutations in SPA genes cause early flowering under short day but not long day conditions, indicating that the SPA gene family is essential for the inhibition of flowering under non-inductive short day. We further show that early flowering of spa1 mutants is fully dependent on the floral inducer CONSTANS but independent of the photoreceptor phytochrome A. Consistent with the CO-dependent early-flowering phenotype, spa mutants show strongly enhanced FT transcript levels in short day. CO mRNA abundance, by contrast, is not altered in spa mutants, indicating that the observed increase in FT transcript levels is not caused by a change in the level or pattern of CO gene expression. The proteins SPA1 and CO interact in vitro and in vivo, and we therefore propose that SPA1 might be involved in the dark-dependent degradation of the CONSTANS protein.

SPA proteins consist of an N-terminal kinase-like domain, a coiled-coil domain and a C-terminal WD-repeat domain. We conducted a structure/function analysis to determine which domains of SPA1 are important from its function in suppression of seedling photomorphogenesis. Deletion-derivatives of SPA1 lacking the complete N-terminus or part of the kinase-like domain retain SPA1 function in dark- and light-grown seedlings, while deletion of the COP1-interacting coiled-coil domain eliminates SPA1 activity. This suggests that the coiled-coil domain and the WD-repeat domain of SPA1 are sufficient for SPA1 function.

Apart from controlling flowering time and seedling photomorphogenesis, SPA proteins also regulate elongation growth of adult plants. In these three processes, the four SPA genes have overlapping but distinct functions. An analysis of SPA transcript levels suggests that differences in SPA gene expression patterns contribute to divergence in SPA1-SPA4 function. Thus, the regulation of SPA expression could be crucial in the adjustment of plant growth and development to changes in the light environment.

48 Cytosolic Gibberellin-Receptor GID1

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Gibberellins (GAs) regulate various developmental processes in plants. In the last year, we identified a rice *GA Insensitive Dwarf1* (*OsGID1*) gene as for an ingredient of GA-insensitive dwarf mutants by positional cloning and detected the GA-binding activity in its recombinant protein prepared using an *E. coli* expressional system. We studied its ligand-selectivity using some GAs and their derivatives, which made it clear that the affinities to the OsGID1 and their physiological activities are almost parallel and the highest affinity (K_d value) to it was shown to be around 10^{-7}M with GA_4). Three mutated OsGID1s responsible for sever dwarf phenotype lost their GA-binding activity. We also checked the *in vivo* interaction between the GID1 and the negative regulator of GA-signaling, SLR1, using a yeast two-hybrid (Y2H) system. Their GA-dependent interaction was clearly confirmed. Taken together with other informations: (i) endogenous GA carrying physiologically active form accumulated 100-fold more in the mutants compared with that in a wild-type plant, (ii) overexpression of the gene leads to GA-hypersensitive, and (iii) the GID1-GFP was preferentially localized in nuclei, we concluded that the OsGID1 is a rice cytosolic GA receptor.

Secondly, we cloned three genes (*AtGID1a*, *AtGID1b*, and *AtGID1c*), each an ortholog of the *OsGID1* gene, from *Arabidopsis*, and the characteristics of their recombinant proteins were examined. The GA-binding activities of the three recombinant proteins were confirmed by the *in vitro* assay. Biochemical analyses revealed they showed similar ligand selectivity to each other and to OsGID1, and all recombinants showed higher affinity to GA_4 than to other GAs. AtGID1b was unique in its binding affinity to GA_4 and in its pH dependence when compared to the other two. We will refer to the characteristics of the recombinant protein and the *in vivo* experiments using a Y2H system.

49 Investigating The Expression Of The TIR1/AFB Family Of Auxin Receptors: Could miRNAs Hold The Key?

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The plant hormone auxin has myriad roles in plant development. Recently the TIR1/AFB family of F-box proteins have been shown to function as auxin receptors (Dharmasiri et al 2005a). TIR1/AFB1-3 act by binding auxin and, by their involvement within an SCF complex, facilitate the degradation of AuxIAA proteins, negative regulators of the auxin response. The biochemical activity of the TIR1/AFB proteins is well established but little is known regarding the regulation of their expression. Transgenic plants containing $Pro_{TIR1/AFB}$:GUS showed expression throughout the plant (Dharmasiri et al, 2005b). We have generated transgenic plants containing $Pro_{TIR1/AFB}$:TIR1/AFB-GUS and show that the GUS expression in these seedlings is reduced when compared to $Pro_{TIR1/AFB}$-GUS. We are therefore investigating the post-transcriptional mechanisms that control TIR1/AFB expression. One such level of control regulation is by the activity of miRNAs. Arabidopsis contains two miRNA genes (miR393a and miR393b) that have identical sequence to a 21nt region of TIR1, AFB2 and AFB3. In 35S:miR393a plants the level of TIR1 expression is reduced (Navarro et al, 2006) and seedlings are resistant to exogenously applied auxin. GFP expression in $Pro_{miR393a/b}$:GFP lines suggests that these miRNAs are expressed in tissues in which the $Pro_{TIR1/AFB}$:TIR1/AFB:GUS lines lack expression. Furthermore we have investigated the consequences of expressing miRNA resistant forms of TIR1 and AFB2 fused to GFP or GUS. Transient over-expression of TIR1 or a form of TIR1 that is resistant to miR393 results in defects in root development. Therefore we present multiple lines of evidence that expression of the TIR1/AFB auxin receptors is post-transcriptionally regulated in part by miRNA expression.

Dharmasiri et al (2005a) Nature 435, 441.
Dharmasiri et al (2005b) Dev. Cell 9, 109.
Navarro et al (2006) Science in press

50 Cytokinin Regulated AP2/ERF Transcription Factors Are Novel Components of the Cytokinin Signaling Pathway that Function in Concert with Type-B ARRs

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Cytokinin is an essential plant hormone involved in numerous plant growth and developmental processes. Cytokinin signal transduction in Arabidopsis occurs via a multi-step phosphorelay pathway, similar to bacterial two-component phosphorelays, composed of sensor histidine kinases (AHKs), histidine-containing phosphotransfer proteins (AHPs) and response regulators (ARRs). There are two classes of ARRs, type-A ARRs that negatively regulate cytokinin responses, and type-B ARRs that are transcription factors playing a positive role in mediating cytokinin-regulated gene expression. We show that several closely related members of the Arabidopsis AP2/ERF gene family of unknown function are transcriptionally induced by cytokinin through this pathway, and we have designated these Cytokinin Response Factors (CRFs). We have shown that CRF proteins rapidly accumulate in the nucleus in response to cytokinin, and this re-localization is dependent on the AHK cytokinin receptors and the downstream AHPs, but is independent of the both type-A and -B ARR response regulators. Analysis of loss-of-function CRF mutants reveals that the CRFs function redundantly to regulate the development of embryos, cotyledons and leaves. In addition, using microarrays we have determined that the CRFs mediate a large fraction of the transcriptional response to cytokinin, affecting a set of cytokinin-responsive genes that largely overlaps with type-B ARR transcriptional targets. These results indicate that the CRF proteins function in tandem with the type-B ARRs to mediate the initial cytokinin response as novel component of the cytokinin signaling pathway.

51 Structural and Functional Insights into the Regulation of Arabidopsis AGC VIIIa Kinase Family

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The Arabidopsis AGCVIIIa kinase family shares sequence similarity to the PKA, PKG and PKC group of kinases found in fungi and animals. This family contains 17 highly homologous kinases that remain poorly characterized. This is due in part to the observation that with the exception of PINOID (PID), disruption of the corresponding genes has not resulted in mutant phenotypes from which gene function can be inferred, suggesting that these kinases function redundantly. As a first step in identifying the physiological processes controlled by these kinases, we have examined their regulation by post-translational modification and subcellular localization. In animal systems, the activity of AGC kinases is mediated via phosphorylation by the 3-phosphoinositide-dependent kinase (PDK1). We have shown that, similar to their animal counterparts, the majority of Arabidopsis AGCVIIIa kinases are also substrates for PDK1, and that trans-phosphorylation by PDK1 correlates with increased substrate activity. We have shown that PDK1 activates PINOID autophosphorylation and that this activation requires both the C-terminal PDK1 interaction domain (PIF) and an activation loop signature. Using mutational analysis, we demonstrated that although the two conserved regulatory sequences are required for activation, they are not sufficient for substrate specific recognition. We found that plant cell extracts that increase the activity of wild-type PID show a dramatic decrease in their ability to activate a PID PIF-domain mutant. The ability of PDK1 to activate PID in vivo was confirmed by experiments in which PID immunoprecipitated from Arabidopsis cells lacking PDK1 expression, exhibited reduced transphosphorylation activity toward MBP substrate. We find that expression of selected GFP-tagged AGCVIIIa kinases in yeast results in differential sub-cellular protein localization. For two family members, WAG1 and PID, these localization patterns seen in yeast correspond to those observed in planta, suggesting that the interaction partners for these kinases are evolutionarily conserved. Domain swapping experiments were used to identify the insertion domain found in all Arabidopsis AGCVIIIa kinases as sufficient for the specific localization of these proteins. These experiments represent the initial step in a detailed characterization of the role of AGCVIIIa kinases in plant signaling processes.

52 Dramatic and Ongoing Amplification of a Rice Transposable Element

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Despite the widespread prevalence of transposable elements (TEs) in the genomes of higher eukaryotes, what is virtually unknown is how TEs amplify to very high copy numbers without being silenced and without killing their host. In this talk I will report the discovery of rice strains where a TE (mPing) has undergone amplification from ~50 to over 1000 copies within the last century. To determine how a host deals with such a burst of transposition, we characterized hundreds of new insertions and found that 70% were within 5kb of a coding region but that insertions into exons and introns were significantly underrepresented. Further analyses of gene expression and TE activity demonstrate that the ability of mPing to attain high copy numbers is due to three factors: (1) the rapid selection against detrimental insertions, (2) the neutral or minimal effect of the remaining insertions on gene transcription and (3) the continued mobility of mPing elements in strains that already have over 1000 copies. Our results indicate that selection against detrimental insertions occurs at two stages, one rapid and one prolonged. We hypothesize that this prolonged second stage of selection may provide a window of opportunity for potentially adaptive insertions to remain in the population.

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53 Systematic Identification of Cis-regulatory Logic from Microarray Data and Whole-genome Sequence

Michael Beer
Johns Hopkins University

We have developed a systematic approach for inferring cis-regulatory logic from whole-genome microarray expression data.[1] This approach identifies local DNA sequence elements and the combinatorial and positional constraints that determine their context-dependent role in transcriptional regulation. We use a Bayesian probabilistic framework that relates general DNA sequence features to mRNA expression patterns. By breaking the expression data into training and test sets of genes, we are able to evaluate the predictive accuracy of our inferred Bayesian network. Applied to expression data from *S. cerevisiae*, our inferred combinatorial regulatory rules correctly predict expression patterns for most of the genes. Applied to microarray data from *C. elegans*[2], we identify novel regulatory elements and combinatorial rules that control the phased temporal expression of transcription factors, histones, and germline specific genes during embryonic and larval development. While many of the DNA elements we find in *S. cerevisiae* are known transcription factor binding sites, the vast majority of the DNA elements we find in *C. elegans* and the inferred regulatory rules are novel, and provide focused mechanistic hypotheses for experimental validation.

Here we present initial application of this approach to microarray datasets sampling developmental stages and cell-type specific expression in A. thaliana[3].

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(3) Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Schölkopf B, Weigel D, and Lohmann JU. Nature Genetics 37, 501-506 (2005); Birnbaum K, Sasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW, and Benfey PN. Science 302, 1956-1960 (2003).

54 *Or* Encodes a Cysteine-Rich Zinc Finger Domain Containing Protein That Regulates High-Level of β-Carotene Accumulation in Cauliflower

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The *Or* (*Orange*) mutant in cauliflower is a spontaneous, semi-dominant mutation that induces high level of beta-carotene accumulation in various tissues of the plant. A single gene coding for *Or* has been identified using map-based cloning and successfully verified by phenotypic complementation in the wild type cauliflower and *Arabidopsis ap1-1 cal-1* "cauliflower" mutant. Sequence analysis revealed that the mutation is due to a 4.7 kb insertion of a LTR retrotransponson in the *Or* allele, which results in alternative splicing. The *Or* gene encodes a plastid-associated protein containing a cysteine-rich zinc binding domain. The gene appears to be plant-specific. Homologs of the cauliflower *Or* gene were found in divergent plant species, including *Arabidopsis,* tomato, maize, and rice. *Or* is expressed highly in very young leaves, curds, and flowers. The tissue-specific expression was further confirmed by examining the expression of Pro_{or}*:GUS* in transgenic *Arabidopsis* plants. Subcellular location study revealed that OR-GFP targets to leucoplasts in the epidermal cells of young leaves and localized at the plastid division midpoint in developing seeds of transgenic *Arabidopsis* plants. *Or* likely functions in association with chromoplast differentiation for carotenoid accumulation and exerts additional control on plastid division.

55 VirtualPlant: A software platform to support systems biology research in the post-genomic era

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Our long term goal is to understand how internal and external perturbations affect processes and networks controlling plant growth and development. In this project, we start with data integration of the known relationships among genes, proteins and molecules (extracted from public databases and/or generated with predictive algorithms) as well as experimental measurements under many different treatments. We go beyond data integration to conceptual integration by using novel visualization techniques to render the multivariate information in visual formats that facilitate extraction of biological concepts. We also use mathematical and statistical methods to help summarize the data. We implement and combine these approaches in a system we term "VirtualPlant". Whereas our project relates specifically to Arabidopsis, the data structures, algorithms, and visualization tools are designed in a species-independent way. Thus the informatic, math, statistic and visualization tools that we develop can be used to model the cellular and physiological responses of any organism for which genomic data is available.

We have implemented a proto-type that is already being actively and effectively used by enthusiastic beta testers. This tool is being used by biologists and computer scientist alike for the purpose it was designed for - to support the analysis of original genomic data generated by the researchers themselves. We have found that working with experimental biologists, even from very early stages of software development, to be the most effective way to generate real solutions to the problems encountered by researchers in the laboratory. The system will be available from http://www.virtualplant.org (funded by NSF).

56 Establishment and Maintenance of Cell Polarity in Plants

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In plants, more than in other eukaryotes, establishment of cell polarity is one of the major developmental themes. Even fully specified plant cells often retain potential to re-define their polarity. The process of tissue polarization inevitably encompasses *de novo* specification of individual cell polarities in cells within a polarizing tissue. The connection between cellular polarizing events and macroscopic manifestation of polarity such as specification of different cell types along the axis, depend on an action of the signalling molecule auxin and its intercellular directional movement. Polar transport of auxin depends on auxin efflux catalysts of the PIN family, each with specific polar, subcellular localization, which determines direction of auxin flow. The activity of PIN proteins can be regulated at the single cell level by changes in their vesicle trafficking-dependent polar targeting in response to developmental and environmental cues. PIN proteins are not statically localized at their polar plasma membrane domains but show constitutive recycling between the plasma membrane and endosomes, which is dependent on the endosomal regulator of vesicle trafficking - ARF GEF GNOM. The role of this cycling is unclear but it may enable rapid changes in subcellular PIN polarity and feed-back regulation of auxin transport by auxin itself. Despite critical importance of polar PIN localisation for plant development, only little is known about how it is decided to which side of cell PIN proteins will be re-targeted following each internalization step of its constitutive cycling. Available data suggest existence of sequence-specific polar targeting signals and cell type-specific determinants. It seems that central role in the control of apical–basal PIN targeting plays the Ser/Thr kinase PINOID (PID) since polarity of PIN1 localisation in *pid* inflorescence apex is reversed from the apical to basal cell sides. On the other hand, PID overexpression leads to basal-to-apical shift in PIN localization. These data suggest that levels of PID within cells largely contribute to the decision of apical versus basal targeting of PIN proteins, possibly through direct modification of PIN proteins by phosphorylation.

57 Actin-Binding Proteins as Sensors of Cellular Stress

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The cytoskeleton is a key regulator of morphogenesis, sexual reproduction, and cellular responses to extracellular stimuli. Prominent redistribution or alteration of actin filament dynamics occur during attack by fungal pathogens, when guard cells respond to hormonal and light signals, and in the self-incompatibility response of pollen. Changes in actin filament organization and dynamics are often assumed to require actin-binding proteins as stimulus-response modulators, because many of these proteins are regulated directly by binding to intracellular second messengers or signaling phospholipids. In plants, numerous actin-binding proteins have now been identified and characterized; several have novel and unexpected biochemical activities that distinguish them from their non-plant counterparts. Further, all that have been examined bind to and are regulated by phospholipids. Phosphatidic acid (PA) is gaining widespread acceptance as a major, abundant phospholipid in plants that is required for pollen tube tip growth and mediates responses to osmotic stress, wounding and phytohormones. Here we demonstrate that exogenous PA application leads to significant increases in filamentous actin levels in Arabidopsis suspension cells and poppy pollen grains. To investigate further these lipid-induced changes in polymer levels, we analyzed the properties of a key regulator of actin filament polymerization, the heterodimeric capping protein from Arabidopsis thaliana (AtCP). AtCP binds to PA with a Kd value of 17 uM and stoichiometry of ~1:2. It also binds well to PtdIns(4,5)P2, but not to several other phosphoinositide or acidic phospholipids. The interaction with PA inhibited the actin-binding activity of CP. In the presence of PA, CP is unable to block the barbed or rapidly growing and shrinking end of actin filaments. Pre-capped filament barbed ends can also be uncapped by addition of PA, allowing rapid filament assembly from an actin monomer pool that is buffered with profilin. The findings support a model in which the inhibition of CP activity in cells by elevated PA results in the stimulation of actin polymerization from a large pool of profilin-actin. Such regulation may be important for the response of plant cells to extracellular stimuli as well as for the normal process of pollen tube tip growth.

58 In vivo Dynamics Implicate a Role for Dynamin-Related Protein 1C in Polar Cell Growth

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Polarized membrane trafficking is critical to the life of a plant. It is required for both cell division and expansion, including the anisotropic growth of stigmatic papillae, pollen tubes and root hairs. Some proteins, lipid moieties and small molecules influencing polar growth have been identified; however additional factors and their associations that are required for polar, bi-directional membrane trafficking remain to be determined. Members of the Arabidopsis dynamin-related protein 1 (DRP1) family have been shown to have a role in polarized cell expansion and cytokinesis. In particular, DRP1C is required for pollen development and may have a role in polar membrane trafficking during root hair, pollen tube and epidermal cell growth. Using confocal microscopy and fluorescence recovery after photobleaching (FRAP), we show that a functional DRP1C-GFP fusion protein is locaized to the plasma membrane in tips of growing root hairs and expanding pollen tubes, and to the cell plate during cell division. In root hairs, DRP1C-GFP is recruited from the cytoplasm to the lateral root hair tip during periods of fast growth and to the apical tip during periods of slow or no growth, suggesting a post-exocytic function for DRP1C. Pharmacological studies demonstrate that DRP1C-GFP localization at the tips of expanding root hairs is dependent on the actin cytoskeleton, the presence of phosphatidylinositols, calcium gradients, and an active secretory pathway, all of which affect the growth of the root hair. In addition, using a new microscopy technique, variable angle epifluorescence microscopy (VAEM), we discovered that plasma membrane-localized DRP1C-GFP is organized into dynamic and discreet foci in several cell types, similar to mammalian dynamin I during clathrin dependent endocytosis. DRP1C-GFP foci are immobile during their average twenty-eight second lifetime at the plasma membrane and become highly mobile once leaving the plasma membrane. We hypothesize that DRP1C plays a critical role in polar cell expansion in root hairs and in pollen tubes. To confirm DRP1C's role in vivo, we have engineered conditional DRP1C knock-out plants, circumventing the drp1C pollen lethality, which are currently under analysis. In addition, we are using biochemical and microscopy-based approaches to determine the content of DRP1C-positive plasma membrane foci.

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59 Polarazine: a new plant cytokinesis inhibitor

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Descriptions of cytokinesis in plants have typically described a centrally located cell plate ring that expands centripetally to fuse with the parental cell wall creating two daughter cells. Cutler and Ehrhardt (2002) have described an alternate model called "polarized" cytokinesis: this mode of cytokinesis occurs through polar growth of the cell plate across the cell, and occurs predominantly in the large, vacuolate cells of the shoot. An important question is whether the two modes of cytokinesis described, classical and polarized, are mechanistically distinct from one another or processes that use fundamentally the same mechanisms. Here we describe polarazine, a new inhibitor of cytokinesis that we speculate targets a component required for polarized cytokinesis and may therefore suggest that polarized cytokinesis is mechanistically distinct from classical cytokinesis.

Polarazine was identified in a chemical genetic screen for inhibitors of etiolated hypocotyl cell expansion. A systematic cell biological analysis of these inhibitors using GFP-marker lines revealed that polarazine inhibits cytokinesis, inducing cell wall stubs at doses as low as 10 μM. Importantly, its effects on cytokinesis are restricted to shoot cells, a feature predicted of drugs that perturb polarized cytokinesis, since this process occurs predominantly in shoot cells. Live cell imaging of cytokinesis in polarazine treated seedlings reveals that this new compound alters growth of the cell plate in the shoot, consistent with the notion that polarazine's effects are shoot specific.

In addition, polarazine prevents the elongation but not initiation of root-hairs and causes a bias in trichome branching from three to predominately two branches. Thus, polarazine's effects are not restricted to cytokinesis and its target(s) may likely function outside of cytokinesis. Consistent with this, we have found that the length of EB1-GFP comets are reduced is interphase hypocotyl cells.

Analysis of the effects of polarazine on other eukaryotes has shown that it causes embryonic lethality in C. elegans and slows growth of S. cerevisiae. These observations suggest that the target of polarazine may be conserved throughout eukaryotes. Efforts are currently focused on exploiting the activity of polarazine on these organisms to identify its site of action using genetic approaches.

60 Functional Analysis of the Arabidopsis cdc2 Homologue CDKA;1

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In general, the components of the cell cycle control machinery appear to be highly conserved between the kingdoms. For instance, in the genome of Arabidopsis, one cdc2 homologue (CDKA;1) with an archetypical PSTAIRE domain is encoded; CDKA;1 displays about 70 percent amino acid similarity with the human CDK1 and 2. However, due to the specific life style of plants, peculiar mutant phenotypes can be expected. Especially, doomed to be affected in cell cycle mutants is a pronounced haploid life stage in plants, the gametophyte, that generates after a few cell cycle rounds the actual gametes. We have isolated a mutant for cdka;1 and the primary mutant phenotype is a failure to progress through the second mitotic division during male gametophytic development. As a consequence, pollen with only one instead of two sperm cells is produced. This situation leads to dramatic developmental consequences after fertilization since the key event of flowering plants, the double fertilization process, is disrupted. Based on the cdka;1 mutant, we have now started to explore the regulatory context of CDKA;1 in Arabidopsis. We have analyzed the expression pattern and the intracellular localization of CDKA;1. In yeast two hybrid screens we have identified potential novel interactors, and by mutating conserved phosphorylation sites we have addressed the posttranslational regulation of CDKA;1. Our data shows that the molecular mechanistics of CDK regulation is conserved between yeast, animals, and plants. However, the regulatory circuits controlling CDK activity appear to be strikingly different.

61 Energy and Agriculture: The Potential to Use Genetics to Improve the Energy Efficiency of Crop Production

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The production of most crops in the developed world involves the use of a large amount of energy to generate the inputs necessary for food production. These inputs include that needed for the machinery used in activities like planting and harvesting, as well as the large amounts of energy needed for the production of chemical inputs, with nitrogen fertilizer production requiring by far the largest share. The use of large amounts of nitrogen fertilizers has allowed for large increases in yield per acre, but with increased energy prices has become the single largest input cost for farmers growing crops like corn. Further, its use contributes to a variety of environmental problems including the pollution of fresh and marine waters, is the largest source of green-house gases from agriculture and leads to the nitrogen pollution of forests. In order to increase the energy efficiency of crop production, it is necessary to either increase yields while maintaining the level of energy input costs or to maintain yields while decreasing the level of energy inputs. For a crop like corn, the former approach has occurred over the last 20 years due to crop improvements based on breeding selection, the biotechnology traits for insect resistance, improved seed treatments and improved agronomic practice. These have led to increased yields while there has been no increase in the average amount of nitrogen fertilizer used. In order to decrease the level of energy inputs into crop agriculture, the single most important genetic trait would be to increase nitrogen use efficiency, where the level of nitrogen fertilizer would be decreased while maintaining crop yields. We have taken a functional genomics approach of testing a fairly large number of genes by mutation and/or over-expression for their ability to affect growth under moderate levels of nitrogen stress. I will discuss two of these genes, one of which affects growth and one of which affects adaptation to nitrogen stress, as examples of how we plan on trying to utilize the knowledge from Arabidopsis to develop important agricultural crops like corn with improved nitrogen use efficiency.

62 Linking Biomass to Stomatal Development

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Plants are autotrophic organisms that support the life of all animals, including humans. Understanding the mechanisms of plant growth and development provides a foundation to increase biomass and renewable energy resources. As plants grow by capturing and assimilating carbon dioxide, increasing biomass may also help reduces greenhouse gas in the environment. To understand the basic, cell-cell mechanisms of plant growth, we have been studying the developmental roles of ERECTA-family of receptor-like kinases in Arabidopsis. Three ERECTA-family genes show synergistic interaction in regulating cell proliferation during aboveground organ growth. In addition, three ERECTA-family genes have redundant and specific functions during stomatal patterning and differentiation [1]. Specific functions of ERECTA-family genes in preventing excessive asymmetric division and guard cell clustering is achieved via genetic interactions with TOO MANY MOUTHS, which encodes receptor-like protein, and YODA, which encodes MAPkinase kinase kinase [2,3]. Our findings place ERECTA-family genes as regulators of plant growth and stomatal development, two critical aspects of a plant's success and survival.

Although studies have highlighted the importance of cell-cell signaling for proper stomata patterning, factors that positively regulate stomatal differentiation from its meristemoid precursor are not known. The meristemoid possesses a transient, stem cell-like property of regenerating itself through a series of asymmetric division. To understand the mechanism of stem cell differentiation in plant epidermis, we initiated a sensitized genetic screen for stomatal phenotype. We identified a gene, MUTE, in which a loss-of-function mutation leads to arrested meristemoids after excessive rounds of asymmetric division. Map-based cloning determined that the MUTE gene encodes a novel transcription factor. Both loss-of-function and gain-of-function analysis revealed that MUTE is a key regulator of guard cell differentiation. Interestingly, we found that a closely-related paralog of MUTE regulates the initial asymmetric division to enter stomatal development. Take together, MUTE-family genes define positive regulators of stomatal development. How these regulators interact with the cell-cell signaling pathways to accomplish stomatal development is the next exciting question to be addressed.

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[2] Nadeau and Sack (2002) Science 296:1697
[3] Bergmann et al. (2004) Science 304: 1494

63 Genetic analysis of drought tolerance in Arabidopsis

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Drought stress is a common adverse environmental condition that seriously affects crop productivity worldwide. However, deciphering drought tolerance mechanisms has remained a major challenge to plant biologists. To develop new methods to study drought tolerance mechanisms, we searched for novel phenotypes that are conferred by drought stress. In both model plants and crop plants, we identified a root response, termed drought rhizogenesis, as a developmental adaptation to drought stress. Genetic screens using Arabidopsis were devised and the DIG (for Drought-Induced rhizoGenesis) loci that control drought rhizogenesis were defined. Characterization of the *dig* mutants revealed that these mutants also exhibit altered drought stress tolerance, indicating that drought rhizogenesis is intimately linked to drought adaptation of the entire plants and could be used as a trait for researchers to access the elusive drought tolerance machinery. Our work thus established a strategy to directly identify drought tolerance determinants. Molecular cloning of several *DIG* loci revealed that certain chloroplast-targeted proteins are of critical importance to drought tolerance. Furthermore, cell metabolites are found to function as signals in regulating drought stress response and drought tolerance. Our data suggests that that there are multiple mechanisms controlling drought adaptation, which is consistent with the notion that plant drought tolerance is a complex and multigene trait. Discovery of these novel drought tolerance determinants will help us to understand drought tolerance mechanisms and to breed or bioengineer drought resistant crop plants. Supported by USDA-NRI (grant no. 2005-35100-15275 to L.X.).

64 SLIM1/EIL3 Transcription Factor Required for Plant Growth on Low Sulfur Environment

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Sulfur is an essential nutrient required for plant growth. Plants utilize soil sulfate for production of sulfur-containing amino acids that serve as essential dietary sulfur sources for animals. Despite the global nutritional significance of this fundamental metabolic process in nature, regulatory mechanisms of plant sulfur assimilation pathways have never been identified. Plants activate sulfur assimilation for survival on sulfur deficiency (-S). Although numbers of genes that facilitate sulfate transport and assimilatory sulfur metabolisms are known to be induced by -S, the molecular mechanisms controlling the -S inducible gene expression are almost unknown. To identify the key regulators in -S response, we isolated Arabidopsis mutants showing aberrant responses to -S. The expression of SULTR1;2 sulfate transporter was visualized as GFP fluorescence in a transgenic plant expressing SULTR1;2 promoter-GFP fusion gene construct, and this plant was used as a parental line for the EMS mutagenesis. The isolated mutant, sulfur limitation1 (slim1), lacked the -S response of SULTR1;2 gene expression. Microarray analysis of the slim1 mutant indicated that the majority of -S responsive genes, including those for the uptake and internal utilization of sulfate, catabolic sulfur reutilization and for the secondary metabolisms, was regulated by SLIM1. The plant growth on -S was affected by the slim1 mutation, but was complemented by SLIM1 expression. SLIM1 encoded an EIL-family transcription factor, EIL3, but was essentially required for plant growth and sulfur metabolisms on low sulfur. Our results suggested that SLIM1/EIL3 members may function specifically in sulfur response independent of the EIN3-mediated ethylene signaling pathways. SLIM1 was expressed in vascular tissues mainly in roots, and consistent with its function as a transcription factor, SLIM1 protein was localized in the nucleus. Significance of the SLIM1-regulated pathways in -S response will be presented in the conference.

65 Regular Circuitry in Embryonic Stem Cells

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The capacity of embryonic stem cells (ESC) to self-renew and to give rise to virtually all somatic lineages holds much promise for human regenerative medicine. Moreover, ESCs provide a unique opportunity in which to study early development and cell fate decisions. We have begun to determine how transcription factors, chromatin regulators and signaling pathways control the gene expression programs responsible for ESC self-renewal and pluripotency.

The transcription factors Oct4 and Nanog have been shown to have essential roles in the establishment and/or maintenance of embryonic stem cells, but their roles are poorly understood. Polycomb group proteins are essential for early development in metazoans but their contributions to human development are not yet established. We have used genome-wide methods to identify the genes that are controlled by these regulators and to develop a model for the core transcriptional regulatory network in ESC. Our results indicate that these transcription factors and chromatin regulators occupy a special set of developmental genes in ESC that must be repressed to maintain pluripotency and that are poised for activation during ES cell differentiation.

66 Arabidopsis 2010

Machi Dilworth
NSF

Introductory Remarks

67 Arabidopsis 2010: Integrating the Unknown-eome with Abiotic Stress Response Networks in Arabidopsis

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The long-term goal of our 2010 project is to determine the contribution of every gene in Arabidopsis thaliana to a plant's response to abiotic stress. The specific focus is on genes of unknown function and their role in networks that mediate responses to chilling, drought, salt, flooding, high light, and oxidative environments. These stresses are the primary cause of crop loss world-wide. While expression-profiling studies have revealed stress induced expression changes for 1000s of genes, most of these genes have not been characterized for biochemical and genetic functions. Our specific aims include the following: 1) Determine the change in stress tolerance for > 1,600 plant lines harboring disruptions in genes of unknown function. 2) Identify > 20,000 potential protein-protein interactions using a random yeast two-hybrid screening strategy. 3) Over-express in transgenic plants > 150 selected genes of unknown function, and test transgenic plants for changes in stress tolerance. 4) Profile changes in gene expression in > 20 stress-response mutants grown under at least two different stress conditions. 5) Coordinate a centralized website for information on genes of unknown function from Arabidopsis (http://bioinfo.ucr.edu/POND). These specific aims are designed to identify genes of unknown function that make significant contributions to the networks that mediate a plants response to stress.

68 Arabidopsis 2010: Functional Genomics of Cation Transporters

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Transport directly impacts all life processes, including nutrition, reproduction, response to stimuli, movement and adaptation to the environment. Although transporter genes make up nearly 5% of the total genome in Arabidopsis, the functions of most transporters are still unknown. One major goal of this project is to determine the roles of a large family of putative cation/proton exchangers (CPA), including genes of the CHX, KEA, NHX and CAX families. Approaches include determining (i) tissue expression using promoter::GUS, and analyses of whole-genome transcriptomics, (ii) membrane localization using GFP-tagged proteins, (iii) biochemical function by expression in a suitable yeast mutant, and (iv) biological function through analyses of mutants. To facilitate the characterization of plant genes, the Cre-*loxP* site-specific recombination system was adapted to make reporter vectors for plant expression studies. This system allows promoter fragments to be cloned into a small vector (univector) and subsequently recombined *in vitro* with binary vectors containing different reporter genes precisely at near-perfect efficiency. These new vectors are efficient and economical alternatives to the other plant reporter vectors currently available. To integrate transport with the plant life cycle, the first genome-wide analysis of transporters showed that 67% of all classified transporters are expressed in the male gametophyte. Some genes are developmentally regulated during microsporogenesis, and others, including 14 CHXs, are preferentially expressed in pollen. CHXs are also expressed in roots, hydathodes, root tips, and guard cells. Transiently expressed CHXs and KEAs tagged with GFP were localized mostly to endomembranes of onion epidermal cells. All NHXs except NHX7/SOS1 complemented salt sensitivity of yeast *nhx1* mutants while KEAs did not. CHX did not restore salt-tolerance in yeast mutants, but improved yeast growth at low K^+ and alkaline pH, suggesting CHX has a role in K^+ uptake under certain conditions. Single mutants of CHX or KEA showed no obvious phenotype; however, mutants lacking a guard cell CHX showed impaired stomatal opening induced by light. Our results suggest that CHXs and KEAs are functionally distinct from NHXs, and that members of the CHX family have roles in osmoregulation. For resources, tools and mutants available to the community, see http://www.life.umd.edu/CBMG/faculty/sze/lab/2010.html, http://www.cbs.umn.edu/2010/, or http://public2.bcm.tmc.edu/labs/2010/.

(Supported by National Science Foundation Arabidopsis 2010 to HS, KDH, and JMW)

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69 Temporal and Spatial Expression Profiling of the Arabidopsis Response to Powdery Mildew

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In response to pathogens, plants such as Arabidopsis thaliana mount an extensive transcriptional response that includes a myriad of defensive responses including cell wall strengthening, the synthesis of anti-microbial compounds, and the expression of pathogenesis-related proteins (such as PR1). In addition, the signal transduction, regulatory, secretory, and trafficking machinery required for the proper synthesis, transport, and localization of these products is activated or induced. Golovinomyces orontii, a causative agent of powdery mildew, exclusively infects epidermal cells with differential transcriptional and physiological responses occurring in the infected epidermal cell, the neighboring epidermal cell, and the adjacent mesophyll cells of the infected leaf. We are using laser microdissection (LMD) to isolate RNA from these distinct populations of plant cells at a sampling rate and with sufficient time points to capture the underlying biological processes. In order to perform this global temporal and spatial expression profiling using LMD-harvested populations of cells, we have developed or optimized existing tissue preparation, RNA isolation and amplification protocols for downstream use with Affymetrix ATH1 GeneChip analysis. As we are also interested in deriving the regulatory circuitry of this defense response, we are developing new methods to identify known and novel cis-acting regulatory elements from temporally (and spatially) resolved expression datasets. Using a novel approach that does not require a priori clustering of the gene expression data, we have identified single and interacting cis-acting regulatory elements of import. In summary, the temporally and spatially resolved global expression data generated through this work coupled with new methods for the statistical analysis of these data will facilitate a detailed mechanistic understanding of the powdery mildew-Arabidopsis interaction and derivation of the regulatory circuitry associated with this interaction.

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70 Genome-Wide Analysis of Arabidopsis Small RNAs

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Small RNAs [microRNAs (miRNAs), small interfering RNAs (siRNAs), and trans-acting siRNAs (ta-siRNAs)] function as posttranscriptional regulators of genes involved in biochemical processes ranging from cell growth and development, gene silencing and defense responses, to epigenetics. Each type of small RNA forms through a distinct biogenesis pathway. Despite the large number of integral biological processes affected by small RNAs, their accumulation patterns, genomic distribution, abundance and other populations-wide characteristics are not fully understood. We seek to delineate global population characteristics for the endogenous small RNA transcriptome of *Arabidopsis thaliana*. We have developed several computational and statistical methods to analyze genome-wide small RNA patterns in Arabidopsis. With the introduction of highly parallel '454' pyrosequencing and other techniques, the small RNA transcriptome can now be profiled at significant depth. We have used this technique to analyze genome-wide small RNA patterns in silencing-defective mutants to assign functions and redundancies for small RNA biogenesis factors at the genome-wide level.

71 The predicted Arabidopsis interactome

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The complex response of an organism to environmental or developmental stimuli is frequently mediated by interconnected reticulating signal transduction pathways. One of the major signaling mechanisms involves protein-protein interactions between evolutionarily conserved protein partners. High throughput experimental approaches have generated near complete sets of all proteins interactions for yeast, C. elegans, and Drosophila and seeded the field of interactomics. Most eukaryotes share a considerable number of genes, and up to 50% of eukaryotic genomes consists of common orthologs. It is thus possible to predict protein-protein interactions on the basis of finding interacting ortholog pairs or interlogs. This technique has been widely practised in higher animal systems such as humans (the OPHID database). We present here a predicted Arabidopsis interactome based on interlog pairs. Currently, over 17,000 predicted interactions have been identified. Preliminary analysis using OSPREY network visualization software has identified many common signaling pathways. We present this interactome as a first step towards understanding global signaling in Arabidopsis, and to whet the appetite for those who are awaiting the results from systematic high throughput experimental approaches.

72 Polydactyl Zinc Finger Transcription Factors can be used as efficient tools to discover Arabidopsis mutants with enhanced homologous recombination

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We developed a vector system for easy assembly of polydactyl zinc finger (PZF) modules to be used in artificial transcription factors (PZF-ATFs). In vivo performance of differently designed PZF domains, fused with the VP16 activation domain, was tested in yeast strains harbouring a repressed MEL1 reporter gene with different types of multi-finger binding sites on a chromosomal locus. These tests revealed that the most effective trans-activation occurred for combinations of non-interrupted DNA binding sites and PZF domains containing short TGEKP linker sequences between the different zinc fingers. For expression of PZF-ATF proteins in planta, we used the promoter of the ribosomal protein gene RPS5A, which is primarily active in embryonic and dividing tissues. We constructed a library of about 4000 genes encoding three-fingered VP16:PZF proteins and introduced this to near saturation into an Arabidopsis homologous recombination (HR) indicator line. Primary transformants were selected that exhibited multiple somatic recombination events. After PCR-mediated rescue of PZF sequences, reconstituted PZF-ATFs were reintroduced in the target line. In this manner, a PZF-ATF was identified that led to a 200- to 1000-fold increase in somatic HR, also in a different type of indicator line. A mutant plant line expressing the HR-inducing PZF-ATF exhibited increased resistance to the DNA-damaging agent bleomycin and was more sensitive to methyl methanesulfonate (MMS), a combination of traits not described before. Our results demonstrate that the use of ZF-ATF pools is highly rewarding when screening for novel dominant phenotypes in Arabidopsis.

73 Engineering Specific and Efficient Gene Silencing with Artificial microRNAs

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MicroRNAs (miRNAs) represent an endogenous class of small RNAs, which are produced from longer hairpin precursors. They bind to target RNAs, mostly messenger RNAs by complementary base-pairing, and trigger post-transcriptional gene silencing. In plants, cleavage and subsequent degradation of target transcripts has been described as the predominant mode of miRNA action. Most known targets of Arabidopsis miRNAs share high sequence complementarity with their regulating miRNAs. To describe the full spectrum of miRNA target genes, we have previously determined precise sequence parameters of plant miRNA target selection, which allow reliable prediction of miRNA targets.

In the presented study, we have systematically replaced miRNA sequences in the context of endogenous precursors to produce artificial 21mer miRNAs (amiRNAs). These were predicted to specifically silence single or multiple endogenous transcripts in Arabidopsis thaliana, and resembled endogenous miRNAs in several aspects, such varying numbers of target mismatches. AmiRNAs were efficiently processed, and overexpression of most precursors resulted in phenotypes that resembled those of plants with mutations in the respective target genes (one or many) at high frequency. Molecular profiles by microarray analyses indicated transcript silencing of the majority of predicted target genes, and also high specificity for only predicted targets. AmiRNAs were also functional when expressed from tissue specific or inducible promoters, and they were found to act largely cell autonomously. Thus, they make a unique tool for directed gene silencing in plants.

AmiRNAs allow efficient and specific silencing of single or multiple transcripts in a strand-specific fashion. Since they bind only short elements in target transcripts, they might also be engineered to silence specific splice forms, and they can easily function to compare loss of gene activities in different genetic backgrounds. Most importantly, phenotypes induced by amiRNA expression might be complemented by amiRNA resistant versions of the target gene. To facilitate the use of amiRNAs, we have generated a web tool for the automated design of amiRNAs, which is available to the public at wmd.weigelworld.org. The template plasmid pRS300 for PCR directed engineering of amiRNAs in the context of the miR319a precursor is available on request.

This study was supported by the Max Planck Society and the DFG (SFB446).

74 The Receptor-like Cytoplasmic Kinase Gene SHORT SUSPENSOR Regulates Extra-Embryonic Development in Arabidopsis

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Division of the Arabidopsis zygote is asymmetric and generates two daughter cells with fundamentally different developmental fates: while the apical cell produces the spherical pro-embryo, the basal cell forms the suspensor, a stalk of mostly extra-embryonic cells. We have previously shown that the MAPKK kinase gene YODA (YDA) acts as a molecular switch promoting extra-embryonic fate in the cells of the basal cell lineage. Here, we provide evidence that a putative receptor-like cytoplasmic kinase gene, named SHORT SUSPENSOR (SSP), acts upstream of YDA in this signaling event.

Loss-of-function mutations in ssp cause similar but weaker embryonic phenotypes than yda mutations, ranging from reduced suspensor growth to complete absence of a suspensor. Genetic marker analysis reveals a loss of suspensor identity in cells of the basal lineage concomitantly with changes in auxin perception.

Genetic tests support the idea that SSP acts upstream of YDA: the phenotype of double mutant embryos resembles yda single mutants, while ssp-1 embryos can be rescued by a constitutively active variant of YDA.

SSP encodes a predicted receptor-like cytoplasmic kinase of the RLCK II family. Three functional features can be distinguished in the amino acid sequence: an N-terminal myristoylation motif, a central protein kinase domain, and a C-terminal cryptic tetratricopeptide repeat motif. Mutational analysis indicates that kinase activity is not required for SSP function in planta, while the predicted C-terminal interaction domain is essential. Myristoylation of SSP also appears to be essential and targets the protein to the plasma membrane. We propose that SSP acts as a scaffolding factor rather than a protein kinase.

When mapping the original ssp-1 mutation, which introduces a stop codon in the center of the ORF, we observed that the mutant phenotype appears to be influenced by naturally occurring second-site modifiers. We have investigated this possibility by QTL mapping and could identify one major and two minor QTLs with statistically significant impact on the ssp-1 phenotype. These genes might represent new components of the YDA signaling pathway.