## 1 Energizing Arabidopsis

*Chris Somerville*

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During the past 25 years, plant biologists have developed Arabidopsis into a very powerful tool for basic research. However, total federal funding for plant biology is less than 1% of the NIH budget. This ridiculous situation is not improved by having special interest groups within the plant biology community fighting for crumbs. I have long-believed that the heart of the problem is that plant biology is viewed as agriculture. Legislators view the big problem in agriculture as overproduction and do not see the point of further investments in basic research that might cause more overproduction. Additionally colleagues who work on crops species are likely to be first at the federal trough when the arguments are based on improving agriculture. I suggest that a more relevant social context for basic research in plant biology is energy. Although there is enough fossil fuel to meet our energy needs for three to four hundred years, there is broad consensus that continuing to burn fossil carbon will have unpleasant irreversible environmental consequences. World energy demand is approximately 11 TW. Approximately 100,000 TW arrives at the earth from the sun each year. Unfortunately, the energy density is too low to support large-scale use of photovoltaic cells. By contrast, recent studies by DOE indicate that plant biomass can make a significant contribution. However, the species that are suited for biomass production are undomesticated and have not been adapted for this purpose as yet. I will outline some aspects of basic research in plant biology that could have a significant impact on the rate of progress toward greater dependence on renewable sources of energy.

## 2 Composition and analysis of the chloroplast division machinery

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The division of double-membraned chloroplasts in plant cells is orchestrated by a complex macromolecular machine with components positioned on both the inner and outer surfaces of the organelle and in the intermembrane space. The components of the chloroplast division apparatus must be properly assembled and their biochemical activities coordinated across the two envelope membranes to achieve chloroplast division. The long-term goal of our research is to understand the molecular events driving the constriction of the organelle and its separation into the two daughter plastids. Towards this end, we are using a combination of systems and approaches to identify the components of the chloroplast division complex and establish their biochemical functions. Consistent with the cyanobacterial origin of chloroplasts, most of the plastid division proteins we and others have identified thus far are evolutionarily related to cell division proteins found in prokaryotes, and are localized inside the organelle. These include, among others, the tubulin-like FtsZ1 and FtsZ2 proteins, and the J-domain-like protein ARC6, all of which localize to mid-plastid rings in the chloroplast stroma. Recently, we have uncovered several new cyanobacterial cell division genes that may facilitate identification of additional plastid division genes and proteins. We have also identified one plastid division protein, ARC5, which is a member of the dynamin family of GTPases and is localized on the cytosolic surface of the outer envelope membrane. This protein has no immediate counterparts in bacteria. Together, these data indicate that the chloroplast division apparatus is an evolutionary hybrid, comprising components derived from both the endosymbiotic ancestor of chloroplasts and its eukaryotic host.

Supported by the National Science Foundation

## 3 Prevacuolar compartments as proteolytic processing stations for storage proteins in Arabidopsis

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The *Arabidopsis* embryo accumulates two types of storage proteins, the 2S albumins and the 12S globulins, inside protein storage vacuoles (PSV). Both 2S albumins and 12S globulins are synthesized as precursors in the ER, exported to the Golgi apparatus, and accumulated in PSVs after processing of the proproteins. The processing of the storage proteins, which involves the cysteine-proteases VPE (vacuolar protein enzyme) and at least one aspartic protease, has been assumed to occur inside the PSVs. Multivesicular prevacuolar compartments carrying storage proteins have been reported in legume embryos but their exact role in PSV assembly is not clear. We have studied Golgi stacks, Golgi-derived vesicles, and prevacuolar compartments during PSV formation in *Arabidopsis* by means of electron tomography of high-pressure frozen/freeze substituted samples and by immunolabeling techniques in *Arabidopsis*, and by subcellular fractionation techniques in *Brassica napus*. Golgi staks produce two distinct types of vesicles: 130 nm vesicles carrying the 2S and 12 storage protein precursors and 30-40 nm vesicles carrying the precursors of the processing proteases. Subcellular fractionation studies confirm this distribution pattern. Interestingly, the prevacuolar compartments contain both storage proteins and processing enzymes. To assess if the storage proteins contained inside the prevacuolar compartment were the precursors or the mature forms, we raised peptide antibodies specific for propeptide that are removed from the storage protein precursors during the proteolytic processing. The simultaneous immunolabeling of mature forms and propeptides demonstrated that the storage proteins are mostly processed inside the prevacuolar compartments, before reaching the PSV. We propose that the observed compartmentalization in the proteolytic processing is related to the different luminal pH values detected inside prevacuolar compartments and developing PSVs, which would affect the solubility of the storage protein and the activity of the processing proteases. Supported by an Antorchas Foundation grant to M.S.O. and NIH grant GM61306 to L.A.S.

## 4 Auxin inhibits endocytosis and promotes its own efflux from cells

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The plant hormone auxin is a major regulator of plant development implicated in a variety of developmental processes such as organ initiation, directional growth, meristem activity and apical dominance. Auxin is distributed between cells by a polar transport system, which requires asymmetrically localized auxin transport facilitators from the PIN family. Using an inhibitor of subcellular vesicle trafficking - brefeldine A - it was shown that PIN proteins repeatedly internalize and recycle to and from the plasma membrane. In animals, similar subcellular dynamics, termed constitutive cycling, serve as a mechanism to control the subcellular location and thus the activity of proteins. Also signaling molecules including hormones can modulate constitutive cycling, however no such mechanism of hormone action has been demonstrated in plants. Here we show that auxin specifically inhibits the endocytosis of many plasma membrane localized proteins, without visibly affecting other vesicle trafficking pathways in cell. This effect is specific to biologically active auxins and requires activity of the Callosin-like protein BIG. By inhibiting the internalization step of PIN constitutive cycling, auxin increases the levels of PINs at the plasma membrane. Concomitantly, auxin promotes its own efflux from cells by a vesicle trafficking-dependent mechanism. Furthermore, asymmetric auxin translocation during root gravitropism correlates with reduced levels of PIN internalization. Our data imply a novel mode of plant hormone action: by modulating PIN protein trafficking, auxin regulates PIN abundance and activity at the cell surface providing a mechanism for the feedback regulation of cellular auxin transport.

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## 5 A Role for the Actin Cytoskeleton in Hexokinase Mediated Glucose Signaling

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Glucose has a hormone function in addition to its metabolic role in plant growth. The best understood glucose sensor/transducer is Arabidopsis hexokinase1 (AtHXK1) in which the glucose signaling activity can be uncoupled from glucose phosphorylation. However, the cellular and molecular processes by which AtHXK1 transduces a glucose signal remain poorly defined. Here we show that AtHXK1 is localized to mitochondria and that the actin cytoskeleton has an important role in glucose signal transduction. Localization of HXK1 was shown using different approaches: bio-imaging of transiently or stably expressed AtHXK1:GFP showed continuous colocalization with mitochondria under different treatment conditions; and, leaf organelle purification on Percoll gradients followed by western blotting also demonstrated that HXK1 is localized only to mitochondria. Previous studies have shown that plant mitochondria traffic on actin filaments. Disruption of actin filaments by latrunculin-B or cytochalasin-D altered mitochondrial cellular distribution and blocked HXK1 mediated glucose signaling in protoplast transient expresson assays. In contrast, protoplast treatment with oryzalin to disrupt microtubules did not affect glucose signaling. Furthermore, two null, vegetative actin mutants, act2-1 and act7-4, were shown to be tolerant to 6% glucose (which arrests wild type seedling development) and also were unable to carry out HXK1-dependent glucose signaling. Arabidopsis seedlings expressing actin binding domains of hTalin fused to GFP (A. Hardham, ANU) were used to visualize F-actin under different conditions. hTalin:GFP seedlings showed developmental arrest on 6% glucose, with a loss of filament formation. hTalin:GFP seedlings on 0.5% sucrose had normal growth, but when treated with 0.1 M glucose (but not mannitol) showed actin filament disruption within 60 min. This indicates that F-actin modification is an early response to glucose treatment. Moreover, glucose signaling paradoxically can either require a 'normal' actin cytoskeleton (e.g. responses of act2-1) or can be associated with a loss of F-actin (hTalin:GFP seedling response). Interestingly, AtHXK1-CFP transfected into hTalin:GFP leaf protoplasts was localized predominantly to F-actin, rather than mitochondria. One possibility is that an unidentified actin binding protein or even G-actin is involved in HXK1-dependent glucose signaling.

## 6 Signaling by heterotrimeric and extra-large G proteins in Arabidopsis ABA responses

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In mammals, heterotrimeric G proteins, composed of alpha, beta, and gamma subunits, couple ligand perception by G-protein-coupled receptors (GPCRs) with numerous signaling cascades. Humans have over 20 different Galpha subunits, 5 Gbeta subunits, and over 10 Ggamma subunits (1), leading to great combinatorial diversity in G-protein signaling. By contrast, the Arabidopsis genome has only single canonical Galpha and Gbeta subunit genes (*GPA1* and *AGB1*, respectively) and two identified Ggamma subunit genes (*AGG1* and *AGG2*). In addition to GPA1, the Arabidopsis genome has three genes encoding "extra-large" GTP-binding proteins (XLGs) (2, 3). The carboxy-termini of conceptually translated XLGs are homologous to GPA1, while the amino-terminal region of each XLG contains a putative nuclear localization signal and a cysteine-rich region. We are investigating the roles of these four GTP-binding proteins with regard to Arabidopsis ABA responses. Through the use of a knockout mutant approach, organ and cell-type specific roles of GPA1 in ABA signaling have been demonstrated (4), suggesting that coupling of GPA1 to diverse cell-specific effectors may functionally compensate for the lack of additional Galpha proteins in plants (5). We are also evaluating the alternative hypothesis that XLG proteins function as additional Galpha subunits in Arabidopsis.

This research was supported by grants from the USDA (2001-35304-09916 and 2003-35304-13924) and the NSF Arabidopsis 2010 program (MCB-0209694) to SMA.

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## 7 Axis -dependent gene expression in the lateral organ formation

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In order to unveil the signaling cascade which lead region-specific gene expression responsible for axis-dependent organ formation, we are examining expression pattern of three genes working in the early stages of lateral organ development. Lateral organs, such as leaves, young floral buds and floral organs develop from primordia formed at the fixed position in the peripheral region of shoot apical meristem, and are considered to follow three axes; basal-apical, central-marginal, and adaxial-abaxial. The direction of each axis could be fixed in relation to the position of the meristem. FILAMENTOUS FLOWER (FIL), a member of YABBY family, is responsible to the formation of abaxial side tissue and is expressed in the abaxial side of lateral organ primordia, Promoter analysis of FIL showed that the region-specific expression is based on two discrete cis elements: one is responsible to promote expression at both abaxial and adaxial sides, and the other is to repress at adaxial side. On the contrary, PHABULOSA (PHB), a member of homeobox family, is required for formation of the adaxial side tissue, and is expressed at the adaxial side. Recent researches propose that the region-specific expression of PHB is controlled by microRNA which is expressed at the abaxial side. These observations suggest that the two sides in lateral organ primordia mutually control specific gene expression. However, double-staining analysis of FIL- and PHB-expressing regions showed the two regions are partially overlapped, not supporting the simple mutual-repression model. The third gene, PRESSED FLOWER (PRS), a member of homeobox/WOX family, is expressed at the marginal region of lateral organ primordia. But the FIL-PHB overlapping region covers the PRS expressing region. Based on these observations and other ongoing experiments, we are trying to present a model of axes-dependent gene expression and lateral organ development.

## 8 A Mutation in the Arabidopsis ADK1 Gene Affects Root Gravitropism, Columella Morphogenesis and Lateral Auxin Transport Across the Root Tip

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Gravi-stimulation (GS) in plant organs is perceived by specific statocytes that translate the physical stimulus to a productive signal transduction event that ultimately results in the curvature response of the organ with respect to the gravity vector. Few proteins have thus far been shown to function in the early phases of gravity signal transduction in plant roots. A comparative proteomic approach identified adenosine kinase (ADK) to be differentially represented early in response to gravi-stimulation (GS). The fluctuation of ADK protein spot intensity was accompanied by ADK relocalization into the nuclear fraction after GS. Reverse genetics showed that mutation in one of the two Arabidopsis ADK paralogs, ADK1, results in reduced root sensitivity to GS, altered kinetics of root gravitropic curvature and distorted root cap morphology. Interestingly, the putative auxin efflux facilitator PIN3 was expressed in only a subset of columella cells in *adk1-1* mutant roots, and did not relocalize to the new physical bottom of these cells upon GS, as it does in wild type roots. Also, the auxin-responsive DR5-GUS reporter did not reveal the development of a lateral auxin gradient across *adk1-1* root caps upon GS. Together, the data suggest that ADK1 contributes to the control of early phases of gravitropic signal transduction in the root tip, in addition to contributing to root cap morphogenesis.

Mass spectrometry was provided by the UW-Madison

Biotech. Mass Spec. Center and Washington Univ. Mass Spec. Resource with support from the NIH National Center for Research Resources (Grant No. P41RR0954).

This work is supported by NASA: NAG2-1602.

## 9 A lipid transfer protein-like protein, DIR1, is involved in long distance signaling during the development of systemic acquired resistance

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Defense mechanisms in plants, including systemic acquired resistance (SAR), are induced by various biotic stresses. SAR is elicited in response to local necrotizing infections, which induce the production of an unknown long distance signaling molecule. The signal is perceived in distant tissues, resulting in resistance to normally virulent pathogens. A SAR-deficient *Arabidopsis* mutant, termed defective in induced resistance *(dir1-1)*, can receive and respond to this signal, but either does not produce or does not transmit it. The mutation affects a lipid transfer protein (LTP), which may participate in chaperoning a hydrophobic SAR mobile signal to distant tissues. LTPs have a hydrophobic pocket which can accommodate a hydrophobic molecule such as a fatty acid, lysophospholipid, or, speculatively, a hydrophobic peptide. Leaves of transgenic plants with the *DIR1* promoter driving GUS expression displayed strong staining in vascular tissues, with less staining also observed in leaf mesophyll cells. Furthermore, when these plants were infected by *Pseudomonas syringae* pv. *tomato*, the activity of the *DIR1* promoter was decreased, as demonstrated by reduced GUS staining throughout the leaves. Detection of DIR1/LTP in intercellular washing fluids of wild type, but not *dir1-1* plants strongly suggests that the putative N-terminal signal sequence localizes DIR1 to the extracellular space, as its sequence predicts. DIR1/LTP was detected in petiole exudates (enriched for phloem sap) collected from SAR-induced wild type, but not in *dir1-1* plants, supporting the involvement of DIR1/LTP in long distance SAR signaling. Furthermore, in preliminary experiments using transgenic *dir1-1* plants expressing a DIR1/LTP:GUS fusion protein under the control of the *DIR1* promoter, the SAR response was restored, and GUS activity was detected in exudates from SAR-induced plants. Based on these results, we hypothesize that DIR1/LTP is involved in long distance signaling as either the signal molecule or as the chaperone of the long distance signal.

## 10 Identification of O-GlcNAc modification of proteins in several signalling pathways

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Mutant studies indicate that O-GlcNAc modification of plant proteins is involved in circadian regulation and viral pathogenesis, and responses to gibberellin (GA), cytokinin and light. Deficiency of the two arabidopsis O-GlcNAc transferases (OGTs), SEC and SPY leads to gamete and embryo lethality indicating that O-GlcNAc modification is also required for development. To understand more about the role of this modification in plant development and signaling pathways we are investigating the role of O-GlcNAc modification of three proteins. O-GlcNAc modification and DELLA proteins both negatively regulate of GA responses. Deletion analysis of a rice DELLA has identified a serine/threonine (S/T) rich region that regulates its activity. Studies with arabidopsis OGT and DELLA mutants suggest that O-GlcNAc modification of DELLA proteins or a protein downstream of the DELLA proteins is required for suppression of GA responses. *E. coli*-expressed SEC modifies the S/T rich region of a DELLA protein. Genetic studies indicate that OGTs and GIGANTEA (GI) act in pathways involved in circadian regulation and light responses. GI is modified by *E. coli*-expressed SEC. The Plum pox virus capsid protein (PPV-CP) is O-GlcNAc modified. While PPV infects wild type, spy and sec plants, the infection spread is slower and the virion titer is lower in sec plants. In addition, PPV-CP from sec plants is not O-GlcNAc modified indicating that SEC modifies the capsid. These experiments suggest that O-GlcNAc modification of PPV-CP, GI and DELLA proteins affects the functioning of these proteins. To investigate the effect of O-GlcNAc modification on the activity of these proteins, we are mapping the modified sites, making non-modifiable mutant versions of them and testing the functionality of the mutant proteins.

## 11 Regulation of Flowering Time and the role of Vernalization

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Certain plants, such as biennials or winter annuals, require relatively long periods of cold exposure during winter to initiate flowering the following spring. Cold exposure renders the meristem of such cold-requiring species competent to flower, and this acquisition of competence is known as vernalization. A vernalization requirement ensures that flowering does not occur prematurely before the onset of winter. Our studies of vernalization in Arabidopsis have revealed that meristem competence is a function of the expression level of certain MADS-box genes such as FLOWERING LOCUS C (FLC) that act as repressors of flowering. Exposure to prolonged cold causes an epigenetic switch of these MADS box genes to an unexpressed state, thus rendering the shoot apical meristem competent to flower. This epigenetic switch is caused by covalent modifications to histones of the chromatin of the flowering repressors.

## 12 The control of flowering by day length in Arabidopsis

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In most plants the transition to flowering is controlled by seasonal cues such as changing day length. In Arabidopsis a circadian-clock regulated pathway that promotes flowering specifically in response to the longer day lengths of spring and early summer has been described. This pathway includes the GIGANTEA (GI), CONSTANS (CO) and FT proteins, which act in the vascular tissue of the leaves to promote synthesis or transport of a systemic signal that triggers flower development at the shoot meristem. We have studied how circadian-clock regulation and acute responses to light combine to activate this pathway in response to long days. These processes regulate expression of both *GI* and *CO*, and involve both transcriptional and post-transcriptional regulation. Accumulation of CO, a protein containing two B-box zinc fingers, in the nucleus under long days activates *FT* transcription, and thus early flowering. The spatial regulation of this pathway, and the molecular mechanisms that confer a flowering response to day length will be discussed.

## 13 Studies on the graft-transmissibility of promotion of flowering by *FT* in *Arabidopsis*

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Photoperiodic induction of flowering requires light perception in leaves, followed by transmission of mobile signal from leaves to the apex, where floral meristems are initiated. However, the nature of the mobile signal has remained unknown.

In *Arabidopsis*, floral pathway integrator *FT* acts mainly in the photoperiod pathway through transcriptional regulation by CONSTANS. *FT* encodes a 20kD protein of the PEBP/RKIP family and is expressed in vasculature of cotyledon and leaf where light is perceived. *FT* transcription is immediately induced in these tissues upon transfer from short-day to inductive long-day photoperiod. Promotion of flowering by *FT* requires the activity of another flowering-time gene *FD* which encodes a bZIP transcription factor preferentially expressed in the shoot apex.

To examine whether the effect of *FT* to promote flowering is transmissible across long distance, we performed micrografting experiments. Using 4-day-old seedlings, two-shoot, 'Y-shaped' grafts were assembled on hypocotyls of *ft-1* recipient. There was no difference in flowering time between *ft-1* self-grafts (*ft-1* grafted onto *ft-1*) and intact *ft-1* control, indicating that graft treatment *per se* does not affect the flowering time of recipient plants. First, we grafted *FT* overexpressor (*35S::FT*) as the scion to the *ft-1* stocks. Flowering time of *ft-1* was dramatically accelerated relative to *ft-1* self-grafts or intact controls. This suggests that effect of *35S::FT* to promote flowering was transmitted to recipient *ft-1* plants. We then grafted transgenic plants which express *FT* by tissue specific promoters to the *ft-1* stock. *SULTR2;1::FT* (specific in phloem) is much more effective than *PDF1::FT* (specific in L1 of shoot apex) in promoting flowering of the *ft-1* stock, although these two transgenic plants themselves had similar precocious-flowering phenotype. Finally we grafted WT onto *ft-1* and observed small but significant promotion of flowering. Results of these and other ongoing experiments will be presented.

Supported by grants from PROBRAIN, CREST of JST, and MEXT.

## 14 *INFLORESCENCE DEFICIENT IN ABSCISSION* Controls Floral Organ Abscission in *Arabidopsis*

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Abscission is an active process that enables plants to shed unwanted organs. Because the purpose of the flower is to facilitate pollination, it often is abscised after fertilization. We have identified a floral organ abscission mutant in *Arabidopsis*, *inflorescence deficient in abscission* (*ida*). *ida* is the first floral abscission mutant characterized which shows a complete lack of floral organ abscission. The *ida* gene encodes a small protein with an N-terminal signal peptide, suggesting that the IDA protein is a ligand of an unknown receptor involved in the developmental control of floral abscission (Butenko et al, 2003 Plant Cell 15: 2296-2307). IDA::GUS reporter lines showed the wild-type *IDA* gene to be expressed specifically in the floral abscission zone during the time of abscission. A single copy IDA::GUS line has been crossed to the ethylene insensitive mutant *etr1-1* to investigate the IDA expression in the *etr1-1* background. In addition we have used molecular markers for chitinase and cellulase to track the abscission process in the ida background. Transient GFP expression showed IDA to be localized in the apoplastic space of onion cells. Stable GFP transformants are currently being investigated to look at GFP-expression in planta. In addition we are working on identifying the receptor, and other genes that are components in the same developmental pathway; by doing both yeast two hybrid and activation tagging. Over expression lines will hopefully give us an indication to whether IDA is involved in regulating the last step of the floral abscission, or inhibiting a repair process during abscission.

Hopefully the identification of proteins interacting with IDA will give us further insight into the regulation of the floral abscission process in Arabidopsis.

## 15 A novel positive signal from the fertilization of the egg cell sets off endosperm proliferation in angiosperm embryogenesis

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Double fertilization of the egg cell and the central cell by one sperm cell each produces the diploid embryo and the triploid endosperm and is one of the defining characteristics of flowering plants (angiosperms). Endosperm and embryo develop in parallel to form the mature seed. However, not much is known about the coordination between these two organisms. Here we present the characterization of a mutant of the Arabidopsis Cdc2 homolog CDKA;1, which confers a paternal effect. In cdka;1 mutant pollen only one, instead of two, sperm cells is produced. Mutant pollen is viable but can fertilize only one cell in the embryo sac, thus allowing a genetic dissection of the double fertilization process. We observed exclusive fertilization of the egg cell by *cdka;1* sperm cells. Moreover, we show that unfertilized endosperm develops, revealing a previously unrecognized positive signal from the fertilization of the egg cell initiating proliferation of the central cell. A similar positive signal can already be found in certain species of lower seed plants (gymnosperms), and thus, might reflect an ancient signal in seed development with implications for the evolution of modern flowering plants.

## 16 From embryogenesis to the vegetative plant

*Gerd Juergens*

**University of Tuebingen, ZMBP, Developmental Genetics**

This overview presents recent advances in vegetative development. Embryogenesis establishes the multicellular organisation of the plant body by a process called pattern formation. Cells adopt specific fates according to their relative position. Pattern formation proceeds along two body axes. The apical-basal axis is partitioned into a series of embryonic structures such as cotyledons, hypocotyl and root, which separate the primary meristems of shoot and root located at opposite ends of the axis. Pattern formation along the radial axis generates the main tissue types such as epidermis, ground tissue (cortex, endodermis) and vascular tissue (xylem and phloem surrounded by pericycle). Following seed germination, the self-maintaining meristems of the seedling produce new shoot (leaves, sideshoots) and root structures. In addition, lateral roots are formed from pericycle cells. During organ development, tissue layers are formed and specific cell types are generated. Towards the end of my talk I discuss new findings from our work addressing cell fate specification in early embryogenesis.

## 17 Two tales of meristems

*Chang Seob Kwon, Dilusha William, Louis Saddic, Meina Lu, Yanhui Su*
**University of Pennsylvania**

We are interested the developmental role of chromatin remodeling ATPases in *Arabidopsis*. In one approach we investigated maintenance of the stem cell population of the shoot apical meristem. The SNF2-subgroup ATPase SYD is important for stem cell pool maintenance during adult development. We demonstrate using genetic and molecular analyses that SYD primarily acts on the stem cell promoting WUS pathway. In this pathway SYD acts upstream of WUS. Furthermore SYD is specifically recruited to the *WUS* promoter *in vivo* suggesting that SYD directly regulates expression of this homeodomain transcription factor.

In a second approach we investigated the molecular basis of the meristem identity transition downstream of LFY. During the meristem identity switch, cells in the peripheral zone stop producing secondary inflorescences subtended by bracts and instead give rise to flowers. We have identified several direct LFY target genes at this stage in development. The five best characterized LFY targets encode transcription factors and putative signal transduction pathway components. I will discuss our current understanding of the biological role of these new meristem identity regulators.

## 18 The *MACCHI-BOU* genes regulate organogenesis together with *PINOID*

*Masahiko Furutani, Masao Tasaka*
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The primordia of shoot organs such as leaves arise from the peripheral zone of the shoot apical meristem (SAM). Similarly, cotyledon primordia are formed in the peripheral region of the embryo apex. Both in the SAM and embryo apex, organ primordia emerge in regions where auxin is highly concentrated. The asymmetrical auxin distribution is established by PINOID (PID), a Ser/Thr kinase, through the regulation of cellular localization of PIN-FORMED1 (PIN1), an auxin efflux facilitator. To identify additional genes involved in this process, we carried out a screen for *pid* enhancers, named *macchi-bou* (*mab*). By screening mutations that caused seedlings without cotyledon development, we identified four loci, *MAB1~4*. All *mab pid* double mutants exhibited defects not only in cotyledon development but also in postembryonic organogenesis, suggesting that the *MAB* genes are involved in organogenesis together with *PID* both in embryonic and postembryonic development. A fraction of all *mab* single mutant seedlings displayed defects in cotyledon number, position and separation. In addition to defects of cotyledon development, *mab1* single mutants displayed defects in root development, *mab2* single mutants exhibited the sterility and the aberrant shoot architecture as bracts develop on the inflorescences, *mab3* single mutants were defective in shoot organ formation, and *mab4* single mutants displayed defects in floral organ development. *mab3* was a novel *pin1* allele. The *MAB1* gene was isolated by map-based cloning and it encodes a mitochondrial pyruvate dehydrogenase E1 beta subunit, which converts pyruvate to acetyl-coenzyme A. Our results indicate that a metabolic pathway involving *MAB1* is engaged in organogenesis. A possible role of the *MAB1* gene for organogenesis will be discussed.

## 19 Phosphatidylinositol signaling is involved in the regulation of root system architecture

*Dana MacGregor, Paul Brannon, Jee Jung, Karen Deak, Jocelyn Malamy*
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Environmental conditions exert a profound effect on root system architecture. In *Arabidopsis thaliana* ecotype Columbia, the formation of lateral roots from lateral root primordia is repressed or significantly delayed under conditions of mild water stress. Using this repressed phenotype, we screened for mutants with aberrant root architectures and isolated *Lateral Root Development* mutants from a population of 10,000 EMS mutagenized seeds. One of these mutants, *lrd2*, exhibits a dramatic increase in its total lateral root length (TOT) both on water stress and water plentiful conditions. Moreover, the root systems of *lrd2* plants are significantly less affected by the water stress conditions compared with wild type. Further characterization has shown the increase in TOT is due to both an increased initiation of lateral root primordia and a significantly higher percent of primordia that develop into lateral roots compared to wild type. We have cloned the *LRD2* gene and it is predicted to encode a type III α1 phosphatidylinositol 4-kinase (PI4Kα1). Our progress in defining the function of PI4Kα1 in this system will be reported. The identification of *LRD2* as a PI4K reveals a novel role for phosphatidylinositol signaling in the regulation of root system architecture.

## 20 DRN and DRN-LIKE of Arabidopsis redundantly control early embryonic patterning through interactions with class III HD-ZIP proteins

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Embryo development and the establishment of a SAM to inititate organogenensis are amongst the most fundamental processes of plant development. *DORNROESCHEN/ENHANCER OF SHOOT REGENERATION1 (DRN/ESR1)*, encodes an AP2 domain-containing protein of the ERF (Ethylene Response Factor) type and is involved in shoot apical meristem development and lateral organ formation. A paralogous gene, *DRN-LIKE* exists and is closely linked to *DRN* on chromosome 1. Insertion mutants for both *DRN* and *DRN-LIKE* genes show fused cotyledon phenotypes at low penetrance, similar to those of the *pin1* and *pinoid* mutants. *DRN* also provides a link with the hormone control of gene regulation in the SAM, being positively regulated by auxin and overexpression causes cytokinin-independent effects. This positive auxin activation is consistent with promoter deletion studies and the location of putative auxin response elements. Combining mutant *drn* and *drn-like* mutant alleles shows differential and unequal contributions in different heterozygote/homozygote combinations, with additional embryo-specific phenotypes, confirming a partially redundant function for both genes in embryo patterning.

To understand further DRN/ESR1 and DRN-LIKE function, a yeast two hybrid screen revealed that DRN interacts with the classIII HD-ZIP protein PHAVOLUTA (PHV) and a basic helix-loop-helix protein (bHLH). Experiments also showed DRN-LIKE interacts with these proteins as well as with other proteins from the HD-ZIP III class also comprised of PHABULOSA, REVOLUTA, CORONA and ATHB8. These interactions involves a novel C-terminal domain specific for this sub-class of proteins and we have evidence of ternary protein complexes involving DRN/ESR1, PHAVOLUTA and bHLH. ClassIII HD-ZIP proteins act redundantly to control aspects of leaf development as well as embryo development. We have confirmed all protein-protein interactions in planta using bimolecular fluorescence complementation and biochemically via coimmunoprecipitation. The functional significance of these interactions has been confirmed by in situ hybridisations showing that DRN/ESR1 and DRN-LIKE are expressed in globular and heart stage developing embryos and have partly overlapping expression domains together with those of PHV, PHB and bHLH. These molecular and genetic data clearly imply that embryo patterning is controlled by the redundant function of at least three classes of transcription factors in different combinations and via novel domains.

## 21 Shedding light on metabolism in the dark

*Alison Smith*

**John Innes Centre**

Plants are the source of most of the organic carbon on earth, and are able to create a vast wealth of different components from carbon dioxide and inorganic nutrients. Demand for the products of plant metabolism will expand and diversify in an unprecedented way in the next few decades in response to population increase, climate change, scarcity of fossil fuels and problems of waste disposal. Although the outlines of many metabolic processes in plants are known, many others remain to be discovered. Even for the processes we know about, we have relatively little understanding of either the mechanisms that integrate metabolism with development and with responses to the environment, or the basis of the enormous variations between organs and species in the way in which carbon is allocated to metabolic pathways. Post-genomic developments are at last allowing us to address these broad questions. Genome sequences reveal the metabolic capacity of the plant, transcriptomics, proteomics and high-throughput assays help us to visualise where and when that capacity is expressed, combinations of forward and reverse genetics allow us to probe function and flux control in unprecedented detail and sensitivity, and the developing science of metabolomics gives us a broad view of the whole metabolic network, rather than the narrow snapshots available before. I will use work from my lab and those of our collaborators to illustrate how these new resources have brought about a radical revision of our understanding of a core metabolic pathway, the conversion of starch to sucrose in leaves in the dark.

## 22 Root Exudation of Antimicrobials Mediates Pathogen

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Using a root infection assay, we found that from a collection of eight *Pseudomonas syringae* strains only one pathovar, *P. syringae pv. tomato* DC3000 (*Pst* DC3000), caused root disease in *Arabidopsis thaliana*. The ability of Pst DC3000 to infect Arabidopsis roots was correlated with the ability of the bacteria to resist the antimicrobial compounds present in the root exudates of Arabidopsis, as well as its ability to block the synthesis/exudation of these compounds. The concentrations of antimicrobials in the root exudates were far higher during infection with non-pathogenic strains than in uninfected plants or in plants infected with the pathogenic strain. Purified antimicrobial compounds were bacteriostatic against the non-pathogenic strains *in vitro* at the same concentrations as those exuded by the roots. Mutants of *Pst* DC3000 in the type three secretion system (TTSS) genes *hrcC* (hypersensitive response) and *hrpL* (hypersensitive response and pathogenesis) elicited the same concentrations of antimicrobials from roots as the non-pathogenic strains. Additionally, we will show that the secretion of compounds by Arabidopsis roots is partially controlled by ABC transporters and that impaired phytochemical secretion in some ABC transporter mutants may account for enhanced susceptibility to *Pst* DC3000. Finally, we will show recent data that suggest that *P. syringae* strains are able to communicate with the roots using volatile and diffusible chemical compounds, and that these bacterial chemicals induce root growth inhibition in Arabidopsis. Understanding the chemical and molecular mechanisms by which Arabidopsis roots communicate with microbes in the soil will improve our understanding of rhizospheric processes.

## 23 High throughput metabolomics for the construction of regulatory networks for plant metabolism

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In the plant kingdom metabolites play a crucial role in many aspects of plant performance. It has been estimated that over 100.000 metabolites can be involved in diverse processes of which many are common for most plant species. This large variety exceeds that of other eukaryotic organisms and it is thought that it would offer plants an alternative to withstand environmental and biotic threats being unable to spatially escape from it.

However often vitally important, for many metabolites quantitative variation is observed between and within plant species. This indicates that at least part of this variation is genetically regulated. Although numerous metabolites have been identified to play crucial roles in a wide variety of biological processes, little is known about their regulation.

Quantitative trait locus analysis makes use of the natural variation present in segregating mapping populations to identify loci regulating the observed variation. In recent years a few studies have demonstrated the genetic regulation of a limited number of metabolites using targeted detection methods. Until now however, large-scale QTL analyses using undirected metabolomics was hampered due to the high number of masses detected and the difficulty in accurately aligning large numbers of chromatograms.

We show the successful detection and alignment of >5700 masses in 400 samples, which were used for genetic analysis of the *Arabidopsis thaliana* L*er*/Cvi recombinant inbred line population consisting of 160 lines. Extracts of seedlings were subjected to LC-MS and the obtained chromatograms were aligned using Metalign. Mean values of the >5700 detected masses were used in QTL analysis identifying significant QTL for >2700 masses. The QTL profiles of these genetically regulated masses could be correlated in many cases, revealing co-regulated masses. Genetic regulatory networks could be constructed where nodes, representing metabolites, are connected if they are commonly regulated. Co-regulation of metabolites implies them to be involved in the same biological pathway or process. We validated our approach with evidence from literature and indicate candidate genes for the regulation of common metabolic pathways.

*Joost Keurentjes is supported by NWO Genomics and CBSG.*

## 24 Arabidopsis mutants that are defective in seed storage reserve deposition and mobilization: *RDM1* encodes the triacylglycerol lipase that catalyses the first step in storage oil breakdown

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Germinating seeds rely on their storage reserves to support growth until they achieve photosynthetic competence. Mutants that fail to deposit these reserves during seed maturation, or are unable to mobilize them after germination, exhibit arrested seedling growth. Providing an alternative source of carbon such as sucrose can often rescue their phenotype. This selection strategy was used to isolate over a hundred arabidopsis *rdm* (reserve deposition and mobilization) mutant lines. The mutants fall into seventeen complementation groups. Eight of these groups appear to be new (*rdm1* to *8*). The remaining lines are allelic to characterized mutants (*wri1*, *pxa1*, *ped1*, *ped2*, *pex5*, *chy1*, *icl*, *mls* and *pck1*).

In arabidopsis oil is the major seed storage reserve, making up ~35% of the seed dry weight. *RDM1*, *2*, *3* and *4* are genes required for storage oil breakdown and when the mutants are grown on sucrose medium for five days they retain more than 90% of their oil. Surprisingly when *rdm1* is sown on soil in the glasshouse it germinates normally and many seedlings become established. In contrast *rdm2*, *3* and *4* behave similarly to existing mutants that are blocked in oil breakdown such as *pxa1* and *ped1*. They have reduced germination frequencies and arrest early in post-germinative growth. *RDM1* has been positionally cloned and the gene product characterized. It encodes the triacylglycerol lipase, which catalyses the first step in storage oil breakdown in germinating seeds.

## 25 The FRO3 ferric reductase plays a vital role in iron homeostasis in Arabidopsis

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The Arabidopsis *FRO2* gene encodes the iron-deficiency inducible Fe(III) chelate reductase responsible for reduction of iron at the root surface; subsequent transport of iron across the plasma membrane is carried out by a ferrous iron transporter (IRT1). We have identified seven additional FRO family members in the Arabidopsis genome and our current studies are aimed at determining the functions of each FRO gene. After iron is taken up by root cells, it is thought that iron is re-oxidized to the ferric form and transported as Fe(III)-citrate via the xylem to the aerial parts of the plant. Fe(III) chelate reductase activity is required for further iron uptake by leaf cells; presumably one or more FROs function at the leaf plasma membrane to reduce iron. We used real time RT-PCR to examine the expression of each FRO gene in different tissues, in response to iron limitation and in response to light/dark treatment. *FRO3* is expressed at high levels in leaves and *FRO3* expression is induced by iron-deficiency in leaves. Expression of *FRO3* also is elevated in light-treated plants as compared to dark-treated plants. Analysis of a *FRO3-KO* line shows that *FRO3* functions in Fe(III) reduction in leaves. Iron accumulation is altered in the *FRO3-KO* line as compared to wild type as is the expression of a variety of genes involved in iron uptake, localization and storage. Our results demonstrate that *FRO3* functions in reduction of iron in leaves and that *FRO3* is essential for maintenance of iron homeostasis in Arabidopsis

## 26 Exploring Chemical Space in the Plant World

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Bioactive chemicals have a long history of helping plant physiologists unravel mechanisms, including those involving: inhibitors of GA biosynthesis, inhibitors of ethylene action, inhibitors of auxin transport, cytoskeleton-disrupting drugs, and inhibitors of GDP-GTP exchange proteins, just to name a few. However, this approach has also met with strong criticism due to the complexities associated with understanding the action mode of compounds at the molecular level. This is one reason why drug companies must advertise the side effects of the drugs they sell. What has motivated biologists to revisit their interest in small molecules? While a little more than ten million pure compounds are known in chemical literature, the potential chemical diversity (defined as the number of unique chemical structures) of compounds composed of carbon, hydrogen, nitrogen, oxygen, sulfur, phosphorous, and the halogens (the organic chemists periodic table) of molecular weight <1000 likely exceeds $10^{60}$. The compounds that have thus far been tested for effects on plants are therefore only a minute fraction of the structural possibilities. The development of combinatorial and automated techniques for synthesizing novel compounds brought forth significant enhancement in the productivity of chemists and makes the likelihood of synthesizing molecular libraries that are representative of chemical space much greater. These advances allow for the identification of chemicals that specifically disrupt a process or the function of a protein. Once these chemicals are identified, we can combine their use with genetic screens to identify genes involved in the same process. The use of unbiased libraries of diverse small molecules will allow plant biologists to discover numerous new bioactive molecules valuable for studying the function of uncharacterized plant genes. Importantly, when combined with Arabidopsis functional genomics, chemical genomics is powerful for the effective and efficient analysis of regulatory networks underlying a specific process. The chemical genomics approach can address loss-of-function lethality and gene redundancy and allow instantaneous, reversible, tunable, and conditional control of a phenotype. Well-characterized bioactive chemicals and their targets identified in Arabidopsis can be used in non-model species to improve agronomic traits and increase crop value.

## 27 Studying Novel Plant Peroxisomal Functions by Bioinformatics and Proteomics

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Our knowledge on plant peroxisomal metabolism is limited to the most abundant enzymes that play a role in photorespiration, fatty acid beta-oxidation and ROS metabolism. We applied a bioinformatics approach to specify the peroxisome targeting signals (PTS) for plants by analyzing semi-quantitatively plant ESTs that are homologous to PTS-targeted plant peroxisomal proteins for the nature of their PTS (Reumann, 2004). Specific PTS peptides were defined for higher plants and applied to screen the Arabidopsis genome for unknown peroxisomal matrix proteins. About 220 and 60 proteins were identified that carry a putative PTS1 or PTS2, respectively. About 80% of these proteins are unknown. Novel non-hypothetical proteins include several enzymes involved in alpha-oxidation of unsaturated fatty acids and branched amino acids, 2-hydroxy acid oxidases as well as NADP-dependent dehydrogenases and reductases. Putative regulatory proteins of plant peroxisomes comprise protein kinases, small heat-shock proteins, and proteases. Bioinformatics information for these Arabidopsis proteins has been compiled in the public database "AraPerox" (Reumann et al., 2004).

The predicted targeting of interesting novel proteins is verfied *in vivo* based on YFP and CFP fusion proteins. Indeed, many enzymes have been shown to be targeted to plant peroxisomes, whereas regulatory proteins often fail to be imported, which is most likely due to transiently and highly regulated protein targeting to peroxisomes upon perception of unknown signals. In a complementary approach, we use *Spinacia oleracea* L. and *Arabidopsis thaliana* L. as model organisms for proteome studies of leaf peroxisomes. The contamination by non-peroxisomal proteins is constantly reduced, and many proteins have been identified, including several predicted from our database "AraPerox". Some proteins seem to be modified post-translationally, whereas others are clearly induced by light and other stress conditions. Attempts are currently underway to identify low-abundance and inducible proteins and to map putative phosphorylation sites.

Reumann, S. (2004) Specification of the peroxisome targeting signals type 1 and type 2 of plant peroxisomes by bioinformatics analyses. Plant Physiology 135:783-800.

Reumann, S., Ma, C., Lemke, S. and Babujee, L. (2004) AraPerox. A Database of Putative Arabidopsis Proteins from Plant Peroxisomes. Plant Physiology 136:2587-2608.

## 28 Studying the plant vacuolar ATPase function through hybrid plant-yeast V-ATPases

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Studying the plant vacuolar ATPase function through hybrid plant-yeast V-ATPases. Moshe Reuveni1 and Patricia M. Kane2. 1Dept of Ornamental Horticulture, ARO Volcani Center, Bet Dagan, Israel; 2Dept. of Biochemistry and Molecular Biology, SUNY Upstate Medical Univ. Syracuse, NY 13210, USA. Vacuolar proton-translocating ATPases (V-ATPases) are ubiquitous proton pumps that acidify multiple organelles in all eukaryotic cells. Organelle acidification is important for protein sorting in the endocytic and biosynthetic pathways, zymogen activation, cytosolic pH and Ca2+ homeostasis, and a number of other fundamental physiological functions. In plant cells, V-ATPases are responsible for acidification of the vacuole and thus critical to the vacuole's ability to accumulate solute and proteins and respond to toxic and osmotic stresses. Vacuolar acidification is linked to some of the most fundamental qualities of different plants, for example, the sour taste of lemon and the color of many flowering plants. V-ATPases are highly conserved multisubunit complexes comprised of the V1 sector, a peripheral membrane complex containing the sites of ATP binding and hydrolysis, attached to an integral membrane complex containing the proton pore, the Vo sector. V-ATPases are composed of at least 14 different subunits, and most eukaryotes encode multiple isoforms of at least one of the V1 subunits in their genomes. These isoforms may help to fine-tune V-ATPase function in different locations or under different physiological conditions. Yeast has emerged as the predominant model system for the study of eukaryotic V-ATPases because of the ease of genetic manipulation of this organism. We are using the available genetic data to replace the yeast (S. cerevisiae) V1 complex with a functional plant V1 complex. Preliminary results suggest that replacement of as many as four of the eight yeast V1 subunits with the corresponding Arabidopsis subunits does not prevent V-ATPase activity. Arabidopsis encodes 3 isoforms for each of 3 different V1 subunits (B, E and G). We are expressing them individually and in combination in the yeast system and characterizing the biochemical properties of the resulting complexes. These experiments will provide unprecedented insight into the basic biochemical properties of plant V-ATPases isoforms as well as the potential regulatory roles of the subunit isoforms in Arabidopsis, in other plants and in other organisms.

## 29 LAPs and DAPs: N-terminal Modifying Enzymes of *Arabidopsis thaliana*

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The specificity and mechanisms of N-terminal modifications of the Arabidopsis proteome are being investigated. N-terminal modifications occur during synthesis, are required for activation or regulation of activity, and are involved with degradation of proteins/peptides – in essence, during birth, life and death of the protein. There are three basic N-terminal modifications that are being addressed: 1) limited proteolysis to remove one to three amino acids; 2) modification of the a-amino group; and 3) side chain-specific changes. These processes represent important fundamental aspects of protein maturation and turnover and potentially of plant gene regulation. We have identified a diverse set of enzymes including 27 peptidases and 11 transferases that have likely roles in these N-terminal modification reactions in plants. Here we report studies on three leucyl aminopeptidases (LAP1, LAP2, and LAP3) and two aspartyl aminopeptidases (DAP1 and DAP2). Expression programs, phenotypes of *LAP* and *DAP* knock-out mutants, and *LAP* and *DAP* promoter activities as evidenced by *LAP:GUS* and *DAP:GUS* transgenic lines will be presented. LAP and DAP enzymes have been over-expressed in *E. coli* and activities characterized. Combinatorial peptide libraries are being used to determine the substrate specificity of these N-terminal processing enzymes.

## 30 Abiotic Interactions with the Environment

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**Dartmouth College**

Plants interact with both biotic and abiotic factors in their environments. Interestingly, there is a temporal aspect to many of these interactions. It is widely recognized that the ability to perceive and respond to environmental stimuli can vary over developmental time; it is less commonly acknowledged the plant's perception of and response to environmental stimuli may also vary with the time of day. That is, perception and response may be gated by the circadian clock. In this overview I will discuss recent progress in plant responses to abiotic environmental stimuli, including conditions such as light, water, salt and temperature, and I will also describe recent examples where these responses are affected by the circadian clock. I will also discuss recent progress in elucidating the mechanisms by which the plant circadian clock responds to environmental stimuli, emphasizing light and temperature. In particular, I will focus on the roles of the *PSEUDO RESPONSE REGULATOR* (*PRR*) gene family in entrainment of the oscillator by both light and temperature. Mutational analysis indicates that the temperature sensing mechanism that provides input to the clock is distinct from that used by the plant to respond to cold stress. Despite the ability of the plant to sense and to respond to temperature steps or pulses, it is well established that the period of the clock is maintained more or less constant across a broad range of temperatures. This is termed temperature compensation, and recent advances in this area will also be discussed. Work in my laboratory is supported by grants (MCB-0343887 and IBN-0316056) from the National Science Foundation.

## 31 Clocks, photoreceptiors and photoperiodism

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Our laboratory is undertaking systems level approaches to understanding circadian clock function in Arabidopsis. The long-term goal is to define the circuitry required to generate robust, physiologically relevant rhythms using a combination of forward genetics with whole-genome approaches in an attempt to understand the network of circuits that are required for the core clock, and how the clock exerts its outputs upon the cell. We are beginning to discover that circadian clocks of both plants and animals are composed not of a single autoregulatory loop but rather of multiple positive and negative interlocking feedback loops. The Psuedo Response Regulator proteins PRR7 and PRR9 appear to define one such loop, which interlocks with the previously described feedback loop between CCA1/LHY and TOC1. Forward genetic screens for circadian mutants are not nearly saturated and a recent screen has identified the ELF4 gene and a novel Myb-like DNA binding protein (LUX) as being critical factors for sustaining circadian rhythmicity under constant conditions. The circadian clock is instrumental in photoperiodic time measurement and previous studies have identified FKF1 as an F-box protein that plays a central role in photoperiodic control over time to flowering. We have performed an interaction screen in yeast to identify potential targets for the FKF1 protein and found a novel Dof class transcription factor (Cycling Dof Factor1, CDF1) that appears to function as a key repressor of flowering. FKF1 is involved in the regulation of CDF1 protein turnover, which contributes to photoperiodism. Finally, light plays a crucial role in the response of plants to their environment and in entraining the circadian clock. We identified a clock-regulated F-box protein (AFR) that appears to play a role in regulating light-signal transduction and have identified a potential target of this F-box protein. In summary, the network architecture of the Arabidopsis circadian system is highly complex so as to provide robustness, multiple opportunities for output control, and several pathways for controlling inputs or environmental entrainment of the oscillator(s).

## 32 Mapping the *Arabidopsis* ionome

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Altering the ability of plants to take up and sequester minerals could have a dramatic impact on both plant and human health. Furthermore, understanding the pathways by which metals accumulate in plants will enable the engineering of plants to either exclude toxic metals or extract them from the soil. We have employed mineral nutrient and trace element profiling, using inductively coupled plasma – mass spectrometry (ICP-MS), as a tool to determine the biological significance of connections between a plant's genome and its elemental profile or "ionome". Our focus is on genes that control uptake and accumulation of solutes, including Ca, K, Mg, P (macronutrients in fertilizer), Co, Cu, Fe, Li, Mn, Mo, Ni, Se, Zn, (micronutrients of significance to plant and human health) and As, Cd, Na and Pb (elements causing agricultural or environmental problems). To date we have analyzed the shoot ionome of approximately 50,000 *Arabidopsis* plants. This includes the completion of a forward genetic screen of over 6000 mutagenized Arabidopsis lines (Lahner et al., 2003 Nat. Biotechnol. 21:1215) and a screen of Arabidopsis ecotypes for variation in the shoot ionome. We have successfully used PCR-based positional cloning, DNA microarray based approaches, and recombinant inbred lines to map over 20 loci that cosegregate with the ionomic traits of interest, and such approaches are allowing the identification of genes involved in regulating the ionome. By varying the concentrations of several nutrients in the soil, we have observed several unexpected alterations in the ionome, including significant differences in the accumulation of macro- and micronutrients in response to changing soil iron levels. In a complementary reverse genetic approach we are also screening the Salk Arabidopsis collection of sequenced T-DNA insertional alleles for genes that affect the shoot ionome. To maximize the value of this ionomics approach, we have developed a searchable online database containing ionomic information on over 22,000 plants [http://hort.agriculture.purdue.edu/Ionomics/database.asp], and this database is being updated regularly.

## 33 What can we learn by monitoring rapid O3-induced guard cell responses in Arabidopsis?

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The importance of stomata in regulating ozone entry into the leaf interior is widely recognized. Also a number of Arabidopsis O3 sensitive mutants and ecotypes have been isolated. However, comparative data for their stomatal responses to ozone and the role of stomata in modifying their ozone sensitivity are scarce. This is probably due to technical complications in measuring gas exchange of this species. We have constructed an eight-chamber whole-rosette ozone fumigation system which enables to monitore O3, CO2 and H2O exchange in different Arabidopsis mutants/ecotypes simultaneously.

We analysed stomatal conductance (gs) of O3-sensitive mutants rcd1, rcd2, rcd3 and ecotypes Col-0, Ler and WS-2, as well as of abscisic-acid insensitive mutants abi1, abi2. This revelad that the initial values of gs were higher in abi1 and abi2 than in Ler. Similarly gs was higher in rcd1, rcd2, rcd3 than in Col-0. Closer inspection of stomatal behaviour right after the onset of O3 exposure revealed, that there was a rapid transient depression of gs in Col-0, Ler and Ws-2, as well as in mutants rcd1, rcd2. The maximum of given initial depression was reached already within 6-8 min of ozone exposure. Interestingly this depression was absent in rcd3, abi1 and abi-2. The presence or absence of this transient depression in gs is not dependent on O3 concentration as a wide range of different concentrations (75 – 450 ppb) were studied. About two hours after the onset of O3-treatment the stomata started to close ultimately in all cases, the closure rates and injury symptoms were different in different mutants/ecotypes.

The concentration of ABA (measured in rcd1, rcd2, rcd3 and Col-0 ) was not induced within 3 h of O3 exposure, indicating that initial stomatal closure was induced by ABA. Still after 8 h a ten-fold rise in ABA was detected in wt as well in all rcd mutants. To address mutual relationships between ozone- and ABA-induced stomatal responses and induction of the oxidative burst, we analyzed ROS (reactive oxygen species) production in leaf tissue at the time of the transient depression in gs and also after the reopening in rcd3 and abi2 and corresponding wildtypes. ROS production was visible by confocal microscopy in the stomatas of Col-0, Ler and rcd1 at the time of transient depression in gs due to ozonation. Whereas the stomatas of rcd3 and abi2 did not show ROS production.

## 34 Two Visual Cycle Homologs, *Ccd8/Max4* and a Putative Short-Chain Dehydrogenase/ Reductase, are Required for Normal Green Light Responses in *Arabidopsis thaliana*

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Light is a critical signal that guides developmental decisions in the dark-grown seedling. Specific quantities and qualities of red, blue and far-red light have well-defined roles in regulating molecular, physiological, and biochemical events during seedling establishment. Effects of green light have been reported and indicate that green light antagonizes aspects of red and blue light mediated development. In our laboratory it has been shown that a short, single pulse of green light opposes the effect of red and/ or blue light regulated stem elongation and plastid transcript accumulation. Genetic studies have shown that the green light signal must be transduced by an unknown photosensory system that requires the beta subunit of the heterotrimeric G-protein. If the sensory system requires a G-protein, it may have other parallels to animal light transduction schemes. Analysis of *Arabidopsis thaliana* genomic sequence revealed the presence of genes homologous to those required for animal vision. These include genes necessary for chromophore synthesis/ modification *Ccd8/Max4* (homologous to RPE65) and a putative short-chain dehydrogenase/reductase with similarity to animal retinal dehydrogenase/reductase (retSDR4). T-DNA insertion mutants of *Ccd8/ Max4*, show defects in known green light responses; possessing short hypocotyls and a failure to down-regulate specific plastid transcripts with pulsed green light. Putative short-chain dehydrogenase/reductase mutants showed an exaggerated advancement of de-etiolation, including short hypocotyls and roots, early hook opening and augmented induction of light-regulated gene expression. The defects in response to green irradiation remarkably parallel the defects observed in the G-beta mutants, suggesting these elements may be intricately related. These studies indicate that plant homologs of three proteins required for rhodopsin-mediated light sensing in animals are required for normal green light sensing in plants.

## 35 The potential of engineering natural products to improve disease resistance in Arabidopsis thaliana.

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Plants are organic chemists *par excellence*, synthesizing a vast number of small molecules that are used for inter plant and inter species communication such as chemical warfare and as attractants for pollinators. Plants have developed different defence strategies towards their attackers, and pests have similarly designed various pathogenesis strategies for their attacks.

Natural products of the cruciferous plants include the sulphur rich glucosinolates and indole alkaloids. Elucidation of the biosynthetic pathway of glucosinolates has enabled us to explore the potential of engineering specific GS profiles to improve plant disease resistance. We assess disease resistance of the engineered CYP79A2, CYP79A1, and CYP79D2 Arabidopsis plants accumulating high levels of BGS, p-OHBGS or IPGS and MPGS, respectively towards the three plant pathogens *Alternaria brassicicola, Pseudomonas syringae* and *Erwinia carotovora* with distinct pathogenesis strategies. We demonstrate that engineering of specific GS profiles alters disease resistance against specific pathogens either by direct toxic effects or by modulating plant defense signaling.

## 36 A Functional Genomics Approach to Disease Resistance Signaling

*Jane Glazebrook, Raka Mitra, Ingrid Peterson, Masanao Sato, Lin Wang, Fumiaki Katagiri*

**Department of Plant Biology, University of Minnesota**

Plants respond to pathogen attack by activation of a suite of inducible defense responses. Effective defense depends upon rapid activation of these responses. The signal transduction network controlling activation of defense responses is complex. Genetic analysis in Arabidopsis has identified many important components of the signaling network, but it seems likely that many genes with important roles in defense signaling remain to be discovered. Genome-wide expression profiling has been used to identify genes that show expression changes in response to pathogen attack. It is known that several genes that are important in defense show increased expression in response to pathogen attack, so the set of genes that are induced in response to pathogen attack may include some that are important for disease resistance. This idea is being tested using the bacterial pathogen *Pseudomonas syringae* and the fungal pathogen *Alternaria brassicicola*. Mutants with defects in pathogen-induced genes are isolated from public collections of T-DNA insertion mutants and tested for enhanced susceptibility to these pathogens. Genes that are found to be important for resistance will be studied using a small custom microarray. This array can be used to query a set of pathogen-responsive genes that represents many different patterns of pathogen-responsive gene expression. Characterization of mutants using this array should allow mutants with defects in signaling to be identified. The altered patterns of gene expression in signaling mutants should reveal their roles in the signaling network.

## 37 *Pseudomonas syringae* manipulates systemic plant defenses against pathogens and herbivores

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Many pathogens are virulent because they specifically interfere with host defense responses and therefore can proliferate. Here, we report that virulent strains of the bacterial phytopathogen *Pseudomonas syringae* induce systemic susceptibility to secondary *P. syringae* infection in the host plant *Arabidopsis thaliana*. This systemic induced susceptibility (SIS) is in direct contrast to the well studied avirulence R gene-dependent resistance response known as the hypersensitive response that elicits systemic acquired resistance. We show that *P. syringae*-elicited SIS is caused by the production of coronatine (COR), a pathogen-derived functional and structural mimic of the phytohormone jasmonic acid (JA). These data suggest that SIS may be a consequence of the previously described mutually antagonistic interaction between the salicylic acid and JA signaling pathways. Virulent *P. syringae* also has the potential to induce net systemic susceptibility to herbivory by an insect (*Trichoplusia ni*, cabbage looper), but this susceptibility is not caused by COR. Rather, consistent with its role as a JA mimic, COR induces systemic resistance to *T. ni*. These data highlight the complexity of defense signaling interactions among plants, pathogens, and herbivores.

AB and JC contributed equally to this work.

## 38 Efficient discovery of regulatory loci in plant defense by exploitation of natural variation

*Remco Van Poecke, Lisa Lenarz-Wyatt, Fumiaki Katagiri*

**University of Minnesota**

Plants are exposed to a broad range of biotic attackers, such as microbial pathogens and insect herbivores. To defend themselves against these attackers, plants have evolved many different, often inducible, defense mechanisms. Previously, I've been working with known mutants to get a better understanding of signaling networks underlying induced plant defense. This work focused on mechanistic and ecological aspects of plant defense signaling. In my current project, I focus on the genetic foundation of plant defense signaling networks, more specifically on discovery of new components of these networks. Identification of such components mainly relies on phenotypic characterization of genetic variants. An important source of genetic variation is variation among natural populations (accessions). This resource has so far been underexploited. The main reason for this is the lack of rapid, sensitive methods to survey this variation and isolate the corresponding genes. A major problem is the separation of the different genes affecting the variation between two accessions. Currently, I am working on novel method to efficiently identify new genes involved in plant defense signaling by taking advantage of natural variation among Arabidopsis thaliana accessions. The method aims at rapid separation of the genes underlying this variation using DNA micro-array expression profiles and mapping of the genes on the genome using Affymetrix Genechip. Subsequent cloning of the genes will allow detailed characterization of these genes. Development of this sensitive and efficient method will be a substantial contribution to the elucidation of plant defense signaling networks and to plant systems biology in general.

## 39 Genetic and Epigenetic Mechanisms

*Andy Cal, Anne Hall, Sarah Hall, Song Luo, Bonnie Scott, Jay Shrestha, Daphne Preuss*
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In this session, I will first summarize recent advances in the understanding of genetic and epigenetic mechanisms in Arabidopsis thaliana. I will also review recent studies from my laboratory that highlight the structure and function of centromeres in Arabidopsis and some of its closest relatives. Centromere functions are highly conserved across all eukaryotes, yet there is a notable lack of centromere DNA sequence homology. The formation of new species can be accompanied by a change in chromosome number, often a consequence of the genome reorganization that follows the hybridization of two species. To provide resources for assembling physical maps of centromeres across the Brassicaceae family, we generated BAC libraries in Arabidopsis arenosa, Capsella rubella, Olimarabidopsis pumila, and Sysimbrium irio (available from Amplicon Express, Pulman, WA). In the Brassicaceae family, chromosome number has changed several times, resulting, in Arabidopsis thaliana, in the creation of some centromeres and the elimination of others. These events were accompanied by the rapid divergence of the population of centromere satellites. By analyzing the satellite content of individual BACs, we showed that the rates of satellite array homogenization across a genome are most rapid over local regions, less rapid within a chromosome, and least efficient across the genome. Even so, plant satellite populations can diverge within 10 million years to forms that entirely lack sequence similarity, a rate of change that is more rapid than that observed previously in primates. We also have compared paracentromeric regions from multiple species, demonstrating that Arabidopsis thaliana, unlike its close relatives, has undergone extraordinary expansions. Despite these dynamic changes, centromeres remain functional. To more readily assess the mechanisms that contribute to centromere activity, we have introduced various centromere DNA fragments into Arabidopsis thaliana, with the goal of forming both dicentric and autonomous chromosomes. Assessment of the function of centromere DNA, derived from a variety of species and introduced into Arabidopsis thaliana, is being assessed. In addition, the interaction of heterologous centromere binding proteins with A. thaliana centromere DNA and with heterologous centromere DNA inserted into the A. thaliana genome is under investigation. These studies will clarify the species-specific requirements for centromere activity and will further define the features required to nucleate centromere function.

## 40 Role of RNA polymerase IV in siRNA-mediated DNA methylation and heterochromatin formation

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All eukaryotes have three nuclear DNA-dependent RNA polymerases (RNAPs), namely pol I, II and III. Interestingly, plants express genes encoding catalytic subunits for a fourth nuclear polymerase, pol IV, which does not functionally overlap with pol I, II or III and is non-essentiaI. Disruption of the pol IV catalytic subunit genes, RPD1 or RPD2 inhibits heterochromatin association into chromocenters and causes cytosine methylation to be decreased at pericentromeric 5S gene clusters and AtSN1 and AtSN2 retroelements. The loss of CG, CNG and CNN methylation in pol IV mutants implicates a partnership between pol IV and DRM, the methyltransferase responsible for RNA-directed de novo methylation. Consistent with this hypothesis, 5S gene and AtSN1 siRNAs are essentially eliminated in pol IV mutants. Developmental abnormalities that arise in pol IV mutants include delayed flowering and defects in floral organ identity. The data suggest that pol IV helps produce siRNAs that target de novo cytosine methylation events that are required for facultative heterochromatin formation and higher-order heterochromatin associations, thereby directly or indirectly influencing the expression of developmentally regulated genes.

## 41 Mutational and TAP tag-assisted proteomic analyses and inducible RNA interference reveal the role of the Arabidopsis exosome in embryo/endosperm identity and imprinting, functional specialization of its subunits, and novel RNA substrates

*Sergei Reverdatto[1], Julia Chekanova[1], Nikolai Skiba[2], Jose Alonso[3], Vladimir Brukhin[4], Joseph Ecker[5], Ueli Grossniklaus[4], Dmitry Belostotsky[1]*

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Exosome is an evolutionarily ancient macromolecular machine composed of multiple hydrolytic and phosphorolytic exoribonucleases as well as additional, auxiliary factors. Yeast exosome was postulated to play a pivotal role in numerous and mechanistically distinct reactions of stable RNA processing, cotranscriptional mRNA proofreading, as well as mRNA degradation. The consequences of exosome depletion have not yet been investigated at the whole-organism level in any of the multicellular species of life.

We have affinity-tagged the Arabidopsis exosome in vivo using the TAP technology and will present its proteomic analysis. We will also present the characterization of several exosome subunit mutants. Remarkably, we find that the loss of the RRP4 subunit leads to an ectopic activation of the endosperm-specific markers in the embryo, loss of parental imprinting, and embryo lethal phenotype. In a stark contrast, the loss of RRP41 results in an arrest of the female gametophyte development. This is the first evidence that the individual subunits of the exosome core have specialized developmental functions.

We have also established a novel genetic depletion system for the RRP4 and RRP41 Arabidopsis exosome subunits using inducible RNA interference (iRNAi). This approach helped reveal the essential requirement for the Arabidopsis exosome at the stages of plant life cycle beyond reproductive development, its role in ribosomal RNA processing, as well as a novel function in processing and/or degradation of the RNAP III transcripts.

Supported by grants from USDA and NSF to D.A.B.

## 42 Monitoring transgene silencing in Arabidopsis - a broadly applicable, non-invasive and sensitive system

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Pronounced variability of transgene expression and transgene silencing are commonly observed among independent plant lines transformed with the same construct. The comprehensive study of single-copy T-DNA lines harbouring reporter genes of various kind and number under the control of a strong promoter showed that transcript level mediated silencing effectively accounts for the pronounced transgene expression variability seen among transformants (Schubert et al., 2004). These findings are immediately relevant to all over-expression studies in Arabidopsis and to studies that exploit gene fusions, e.g. for localisation. Moreover, the data indicate that a sensitive monitoring system suitable to identify cells exhibiting transgene silencing would allow for an efficient screening of plants showing stable transgene expression. The finding that the silenced state of a transgene is transferred to different transgenes sharing sequence homology in the transcribed region was exploited to develop a non-invasive monitoring system for the detection of transgene silencing in Arabidopsis. The monitoring system is capable to distinguish between transgenic plants showing stable expression from those that display post-transcriptional gene silencing in a highly efficient manner.

Schubert, D., Lechtenberg, B., Forsbach, A., Gils, M., Bahadur, S. and Schmidt, R. (2004) Silencing in Arabidopsis T-DNA transformants – the predominant role of a gene-specific RNA sensing mechanism versus position effects. Plant Cell 16, 2561-2572.

## 43 In Vivo Protein-Interaction Assays Based on Bioluminescence Resonance Energy Transfer (BRET)

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Genome-scale protein interaction maps are now emerging in several species from experimentation based on proteomic and molecular genetic interaction techniques. Interpretation of these data calls for an ability to monitor protein-protein interactions in live cells and in a signal-dependent fashion. Suitable assays have been put into practice using physicochemical concepts such as resonance energy transfer, fluorescence complementation, split-enzyme complementation, fluorescence enhancement of microinjected probes, and others. We have explored the potential of bioluminescence resonance energy transfer between hybrid proteins tagged with Renilla luciferase and yellow fluorescent protein (BRET, Xu et al., PNAS 96, 151-156 [1999]) to visualize protein-interactions in plant cells. A versatile set of traditional and Gateway-based recombination cloning vectors was constructed for the transient and stable expression of BRET-tagged proteins in planta. With the goal of detecting signaling events in the Arabidopsis light signaling network, we have begun to characterize the potential real-time in vivo interactions of a substantial number of light regulatory gene products. For example, using BRET, it was possible to dissect the juxtaposed motifs governing the nuclear exclusion and dimerization of the Arabidopsis repressor of photomorphogenesis, COP1. Progress in tagging Arabidopsis genes under the control of their authentic regulatory elements and in extending BRET from a cuvette-based assay to the imaging level will be presented. The BRET assay has theoretical and practical advantages, as well as limitations, over competing techniques. It is easily implemented and is a user-friendly and robust technique for monitoring the interaction between two specific proteins in a variety of live plant cell types. Funded by NSF MCB-0114653.

## 44 Isolation of TAP-tagged Protein Complexes From Plants

*Michael Fromm*
**University of Nebraska**

Twenty protein kinase genes have been TAP-tagged, transformed into transgenic rice plants and analyzed for interacting proteins in the T1 plants. Protein complexes have been identified for some but not all of the tagged proteins. The key learnings and results of the technology and biology will be presented, as well as suggestions for successfully using this technology.

## 45 Using metabolomics and transcriptomics data to study metabolic networks in Arabidopsis

*Vladimir Shulaev*

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Metabolomics is an emerging technology that along with other genomics platforms (i. e. transcriptomics and proteomics) and integration of experimental results through mathematical modeling is increasingly being used as a systems biology approach to study the topology and dynamics of metabolic and signaling networks in living systems. Our research is focused on developing methods for high throughput metabolite profiling and application of metabolomics to study stress response in plants using Arabidopsis as a model. We employ a combination of gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) for both targeted and non-targeted metabolite profiling of wild type and mutant Arabidopsis plants subjected to various environmental stresses including heat, drought and oxidative stress. We will present metabolite profiling results for several Arabidopsis mutants and discuss how metabolomics data can be used in combination with transcriptomics and bioinformatics tools to study the biochemical networks involved in Arabidopsis response to a particular environmental stress.

## 46 Metabolomics and proteomics in *Arabidopsis thaliana*– transitions from pattern recognition to biological interpretation

*Wolfram Weckwerth*

**Max-Planck-Institute of Molecular Plant Physiology**

Metabolomics has proven to be superior for pattern recognition analysis of biological samples than any other post-genome technology. Owing to an amplification of metabolic processes reflected on the level of metabolite concentrations and fluctuations a very rapid sample classification is possible. A cluster of multivariate data mining tools is emerging enabling the biological interpretation of sample patterns (Komponenten-Analyse) and the identification of responsible metabolite markers (Loadings). A step further is the complementation with "orthogonal" protein, phosphoprotein and external (environmental) data. These integrative analyses give a higher level of observation of molecular networking in living systems, and, thus, allow the identification of multiple biomarkers embedded in dynamic correlative networks. The techniques for metabolite, protein and phosphoprotein profiling are presented and their limitations are discussed. Based on recent studies the improvement of systemic analysis in *Arabidopsis thaliana* using integrative metabolite-protein profiling is demonstrated.

## 47 Adaptation and variation in Arabidopsis flowering

*Michael Purugganan*

## 48 Linkage disequilibrium mapping in Arabidopsis

*Sung Kim[1], Keyan Zhao[1], Maria Jose Aranzana[1], Erica Bakker[2], Katrin Jakob[2], Clare Lister[3], John Molitor[1], Chikako Shindo[3], Chunlao Tang[1], Brian Traw[2], Honggang Zheng[1], Fengzhu Sun[1], Joy Bergelson[2], Caroline Dean[3], Paul Marjoram[1], Magnus Nordborg[1]*

**[1]University of Southern California, [2]University of Chicago, [3]John Innes Centre**

As part of the NSF 2010 Program, we have carried out a genome-wide survey of polymorphism in *Arabidopsis thaliana* by re-sequencing about 1,500 short fragments in a sample of 96 accessions. The study yielded a very good picture of the pattern of polymorphism in this species. Although *A. thaliana* is a selfing weed, the pattern of polymorphism in general agrees with what is expected for a widely distributed, sexually reproducing species. Linkage disequilibrium (LD) decays rapidly, within 50 kb. Variation is shared world-wide, although population structure and isolation by distance are evident. The data fail to fit standard neutral population genetics models in several ways, rendering the usual "tests of selection" meaningless.

The main motivation for the study was to investigate the feasibility of genome-wide linkage disequilibrium mapping in this species. Because linkage disequilibrium in general decays faster than predicted, within 50 kb, the marker density generated by our re-sequencing study is not sufficient to have truly genome-wide coverage. Furthermore, we find that population structure typically induces very high rates of false positives. Nonetheless, we are able to map several previously known loci involved in variation for flowering time and disease resistance, thus demonstrating the potential utility of the approach.

We argue that future genome-wide association studies must employ higher marker densities, and much larger samples of accessions.

## 49 Evolution of ecologically important traits in relatives of Arabidopsis

*Thomas Mitchell-Olds, Juergen Kroymann, Maria Clauss, and Andrew Heidel*
**Max-Planck Institute for Chemical Ecology, Germany**

What evolutionary factors influence natural genetic variation in plant populations? Building upon earlier studies in Arabidopsis thaliana, we examined genetic variation for insect resistance and secondary metabolism in perennial Arabidopsis lyrata. Glucosinolates and trichome density were significantly heritable, as was resistance to some insect herbivores. Within and between populations, significant natural genetic variation for side-chain elongation in aliphatic glucosinolates allow us to target the methylthioalkylmalate synthase (MAM) locus as a candidate for future experiments in "reverse ecology."

In this same genomic region, A. thaliana harbors complex balanced polymorphism influencing growth rate. We fine mapped phenotypic effects segregating within a one centiMorgan chromosome interval for which lines with mapped recombination breakpoints were available, and examined the sequence signature of historical polymorphism. We found two epistatic growth rate QTL within 210 kb, suggesting a massively polygenic architecture of quantitative variation. One QTL was delimited to a single gene, which shows a nucleotide signature of balancing selection, and whose phenotypic effects are reversed depending on genetic background. If this region typifies many complex trait loci, then nonneutral epistatic polymorphism may be an important contributor to genetic variation in complex traits.

## 50 Pronounced Expansion of Transcription Factor Families in Plants

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Transcription factors (TFs) are central to the regulation of gene expression and are usually members of multigene families. In plants, they are involved in diverse processes ranging from developmental controls to elicitation of defense and stress responses. To investigate differences in expansion patterns of TF gene families between plants and other eukaryotes, we first used Arabidopsis TFs to identify TF DNA binding domains. The Hidden Markov Models of these DNA binding domains were then employed to identify related sequences in rice, Chlamydomonas and 20 other eukaryotic genomes.

Not surprisingly, we found that all Arabidopsis and rice TF families are larger than those in Chlamydomonas. In addition, among 19 families that are shared between animals and plants, more than 14 are larger in plants than in animals. After examining the lineage-specific expansion of TF families in 2 plants, 8 animals, and 2 fungi, we found that plant TF families have undergone much more dramatic expansion compared to those in other eukaryotes. This elevated expansion rate in plants is not simply due to higher duplication rates of plant genomes. Compared with plant genes involved in other functions, TFs have a significantly higher retention rate of duplicate genes. Interestingly, we found that the degree of lineage-specific expansion in Arabidopsis is correlated with that in rice. This pattern of parallel expansion is much more pronounced than the whole genome trend, suggesting the presence of common selection pressure acting on some TFs in both the Arabidopsis and rice lineages.

The high rate of retention among plant TF genes and their propensity for parallel expansion suggest frequent adaptive responses to selection pressure common among higher plants.

## 51 Developmental mechanisms underlying fruit diversification in Brassiceae (Brassicaceae)

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Great variation in fruit structure is observed within the close phylogenetic vicinity of the model species *Arabidopsis*, particularly in the tribe Brassiceae. Members of this clade exhibit fundamental differences relative to the typical silique of *Arabidopsis*, including indehiscence (meaning that seeds are not released) and the presence of a novel structure known as the joint that fully bisects the fruit laterally into two heteromorphic segments. The joint often represents an abscission zone, which allows the proximal and distal segments to be dispersed as individual subunits. We focus our studies on two closely related taxa from the Brassiceae, *Cakile* and *Erucaria*, that vary in two critical aspects in fruit morphology: (1) dehiscence of the proximal segment and (2) abscission along the joint. We have conducted detailed developmental and histological studies of *Cakile* and *Erucaria* that have contributed to our understanding of the evolution of this phenotype from an *Arabidopsis*-like ancestor. The genetic pathway controlling dehiscence and establishment of the valve boundary has been well characterized in *Arabidopsis*, providing a framework for studying the genetic basis for the morphological modifications of fruits in the Brassiceae. We are focusing our studies all on six genes known to play crucial roles in the patterning of the valve margin layer in *Arabidopsis*, with an initial focus on the *SHATTERPROOF* (*SHP*) genes. Based on current *in situ* hybridization experiments in *Cakile*, it appears that the indehiscence of *Cakile* fruit may be correlated with a lack of *SHP* homolog expression in the valve margin. These results have the potential to provide greater insight into the complex remodeling of fruit morphology that occurred in the Brassiceae.

## 52 Evolution of mating systems in *Arabidopsis*: from outcrossing to selfing and back

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The mating system adopted by a plant species determines the patterns of genetic variation in and between populations and thus has profound consequences for the rate and mode of evolutionary change. The genus *Arabidopsis* provides ideal material for analysis of the evolution of mating systems. It includes both self-fertilizing species, such as *A. thaliana*, and obligate out-breeders, such as *A. lyrata*. Furthermore, out-crossing in this genus, as in other crucifers, is due to the operation of a well-characterized self-incompatibility (SI) system, which prevents self-fertilization by inhibiting the hydration and germination of self-related pollen at the surface of the stigma epidermal cells. The genetic determinant of SI specificity is the *S*-locus complex, and inhibition of self pollen is effected by allele-specific interactions between two highly polymorphic proteins encoded within this locus: the S-locus receptor kinase SRK, which is localized on the stigma epidermal surface, and its ligand, the S-locus cysteine-rich protein SCR, which is located in the pollen coat. The SRK-SCR interaction triggers a poorly understood signal transduction pathway within the stigma epidermal cell that culminates in the inhibition of self pollen.

The switch from out-crossing to selfing in crucifers is often associated with loss or inactivation of *SRK* and *SCR* genes, demonstrating that these genes are the primary determinants of the out-crossing mating system in this family. The SI trait can be transferred into the self-fertile *A. thaliana*, which lacks functional alleles of these genes, by transformation with an *SRK-SCR* gene pair from *A. lyrata*, but *A. thaliana* ecotypes differ in their ability to express the SI trait. A study of the mechanism and evolution of self-recognition in out-crossers and its breakdown in selfers can therefore draw on two reservoirs of variation provided to us by nature. On the one hand, the extensive polymorphisms of SI genes may be used to identify the specificity-determining residues or domains in the SRK receptor and its SCR ligand, understand how these two proteins co-evolve to maintain their interaction, and possibly explain how the large SI recognition repertoires were generated. On the other hand, the natural variation in expression of SI in transgenic *SRK-SCR* plants belonging to different *A. thaliana* ecotypes may be exploited to identify the loci that were the targets of selection for self-fertility, understand how the S-locus genes and genes of the SI signal transduction pathway were modified in conjunction with the switch to self-fertility, and thereby further elucidate the mechanism of self recognition and evolution of mating systems in crucifers.

## 53 *Arabidopsis* cytochrome P450 monooxygenases

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Cytochrome P450 monooxygenase (P450s) function in the biosynthesis of lignins, pigments, defense compounds, fatty acids, hormones and growth regulators as well as in the metabolism of herbicides, insecticides and pollutants. The *Arabidopsis thaliana* genome contains 272 P450 genes and pseudogenes that are classified into 45 families (>40% identity) and 72 subfamilies (>55% identity) according to their primary sequence identities. Mutant analysis and expression in various bacterial, yeast and baculovirus expression systems has now assigned function to 39 of these P450s (http://arabidopsis-P450.biotec.uiuc.edu). Towards understanding expression and regulation of these P450s, microarrays containing gene-specific elements for 265 P450s, 40 biochemical pathway markers and 322 physiological function markers have been analyzed for sets of P450s co-expressed in specific tissues and in response to different signaling molecules (JA, SA), fungal defense activators (BTH) and their combinations, plant growth hormones (ABA, IAA, BR), environmental stresses (ozone, UV-B, hydrogen peroxide), etc. These comparisons have detailed the unique and overlapping responses of P450 and biochemical pathway loci to various cues. Analysis of P450 promoter sequences (existing within 2 kb of ATG) co-regulated by individual or groups of stresses has identified specific as well as common motifs that are over-represented among these promoter sets. Annotation efforts have identified a number of unusually long P450 transcripts spanning two adjacent loci that are spliced to produce dimeric P450 proteins containing two heme binding domains or bicistronic transcripts containing complete ORFs for both phase I (P450) and phase II (OMT) detoxification enzymes. Cloning and expression of individual P450 cDNAs in baculovirus and yeast systems have defined substrate binding profiles for a number of fatty acid hydroxylases and, interestingly, demonstrated that the P450 locus coding for HPL in hexenal signaling has been inactivated in the Col ecotype. Molecular modeling of P450s coupled with high-throughput docking approaches are being used to predict potential substrates for P450s with undefined functions for subsequent binding and activity analyses.

## 54 FUNCTIONAL ANALYSIS OF THE UBIQUITIN-PROTEIN LIGASE (E3) FAMILIES IN ARABIDOPSIS

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The post-translational conjugation of one or more Ubs to selected intracellular proteins plays an integral role in numerous growth, developmental, and metabolic processes in plants via its ability to modify the function and/or half-life of its targets. Whereas its main function is to commit proteins for degradation by the 26S proteasome, other functions have become apparent more recently, including roles in DNA repair, lysosomal catabolism, intracellular trafficking, and the regulation of transcription. Among the enzymes responsible for Ub conjugation, the Ub-protein ligases (or E3s) are the crucial elements that control both target selectivity and the nature of the Ub linkage. During our current NSF *Arabidopsis* 2010 project, we discovered that *Arabidopsis* may contain over 1,300 E3 components, making this collection one of the largest functional groups in this plant (~5% of the proteome). Surprisingly, this prediction far exceeds those in animals and fungi, suggesting that plants have specifically expanded the pathway. Here we report our progress in annotating the various E3 families, and defining their functions and targets using biochemical assays, protein interaction screens, expression studies, forward and reverse genetics, and proteomic strategies. Recent successes include the identification of large RING, F-Box and BTB protein families that provide the target specificity to E3 complexes, and the discovery that specific isoforms regulate *Arabidopsis* development and the response to light, ethylene and auxin signals. In fact, the biochemical analysis of TIR1 and its relatives demonstrate that a family of E3s actually participates in auxin reception. The results generated by this study will form an essential framework for understanding E3 diversity, help reveal specific functions for each E3 type, and will develop a database of *Arabidopsis* proteins whose abundance and/or functions are affected by Ub addition. All of this work is currently being made available through the web-accessible PlantsUBQ database (http:/plantsubq.genomics.purdue.edu).

## 55 Analysis of the Arabidopis CDPK Superfamily

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The CDPK superfamily comprises seven types of protein kinases. Calcium-dependent protein kinases (CDPKs or CPKs) contain both a protein kinase catalytic domain and a calcium-binding regulatory domain. There are 34 CDPKs in Arabidopsis, which can be divided into four groups based on sequence similarity. In addition there are eight CDPK-related kinases (CRKs), which have degenerate calcium-binding motifs. The two phosphoenol pyruvate carboxylase kinases (PPKs) have only a catalytic domain and the two PPK-related kinases (PPRKs) contain a C-terminal domain of unknown function. Also related to CDPKs are the three types of SNF1-related kinases (SNRKs), which include 38 enzymes. With the exception of certain SNRK2 and SNRK3 kinases, genetic approaches have not yielded much insight into the physiological functions of individual members of the CDPK superfamily. To better understand the function of the family members we 1) used the yeast two-hybrid system to identify interacting proteins and potential substrates, 2) expressed GFP-tagged kinases to determine their subcellular localization, and 3) used mass spectrometry to identify autophosphorylation sites and protein substrates. In a fourth goal of this project we worked with Santa Fe Community College in Gainesville, FL to develop lessons on DNA technology for community college students. Results from this project are posted on the PlantsP website (http://plantsp.sdsc.edu). Selected results will be described.

## 56 Indispensable Genes Required for Seed Development in *Arabidopsis*

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The goal of the *Arabidopsis* SeedGenes Project is to present detailed information on indispensable genes with a knockout phenotype in the developing seed. This project was made possible through a T-DNA insertional mutagenesis program initiated at Syngenta (Research Triangle Park, NC) 8 years ago in collaboration with the Meinke laboratory. NSF 2010 funding was obtained in September 2001 to transfer work to the public sector and to make extensive information on *EMB* genes available to the community. The project database and website (www.seedgenes.org) at present include information on 295 genes and 462 mutants. A recent upgrade of the database is described in the poster by Rosanna Muralla *et al*. The frequency of *emb* mutants obtained to date is consistent with the presence of 500 to 1000 total *EMB* genes in *Arabidopsis*. A major challenge for the future is to identify those genes that have so far escaped detection. Forward genetics becomes less efficient with increasing saturation because a greater proportion of mutants identified represent duplicate alleles of known *EMB* genes. The question then becomes which candidate genes represent the most promising targets for reverse genetics. We have identified several classes of genes that merit further analysis, including those that resemble essential genes in other model organisms, those for which a knockout homozygote cannot be identified, and those that share an interacting protein partner, metabolic pathway, or cellular process with a known *EMB* gene. Examples of initial results obtained from these different strategies will be described. The poster by Michael Berg *et al*. provides more detailed information on the shared process approach in relation to tRNA synthetase knockouts.

Research supported by the National Science Foundation 2010 Program and by the S.R. Noble Foundation (Ardmore, OK).

## 57 Non-mendelian inheritance of ancestral sequences in Arabidopsis.

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We have recently demonstrated that *Arabidopsis* plants homozygous for recessive mutant alleles of the *hothead* locus can inherit allele specific DNA sequence information that was not present in the chromosomal genome of their parents. This process appears to occur throughout the nuclear genome and affects coding, as well as, non-coding regions. In addition to changes in single nucleotide polymorphisms, regions containing small deletions and insertions can also be modified lending further support to the idea that this is a template-mediated process. Furthermore, this extra-genomic sequence cache appears to persist for multiple generations. Based on our findings we propose that this process represents a completely novel template-directed mechanism for the epigenetic inheritance of DNA sequence information. These findings contradict the established laws of classical Mendelian genetics where allelic information contained in the nuclear genome is stably inherited and transmitted from one generation to the next in a predictable manner.

## 58 Asking for Arrays.

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Microarrays and other large-scale transcriptomic technologies are now common and powerful research tools for biology and have become accepted within the scientific community as useful stepping-stones to a variety of downstream analyses and discovery processes.

Mechanisms for the generation, storage, distribution and on-line or local analysis of data provided from array experiments have become less of a frontier activity and are becoming increasingly mainstream. A substantial number of sites have appeared within the Arabidopsis community alone offering intersecting sets of array data.

Many of these public sites provide a broad palette of on-line tools or references and links to appropriate tools for analysis of the data or appropriate advice to help the novice, the casual browser and the power-user.

Most current array sites broadly conform to international standards such as MIAME and provide their data in a variety of formats from simple CD/DVD distribution through FTP, database queries and other online meta-tools through to web services such as BioMOBY. The hosts for these sites also normally advocate the onward distribution of data to each other and to repositories such as ArrayExpress and GEO for the general good of the plant community.

This talk will summarise the sources of some of these data and tools along with examples of the mechanisms by which users can find their way through the sometimes overly rich choice of alternatives without sacrificing the option of user choice.

We would also like to include an appeal for producers and users of array data to liaise with the databases described (or their own local informaticians) so that their data may be included, mirrored or otherwise served to the community in a way that takes advantage of the broadest range of available tools.

Example references: NASCarrays - http://affy.arabidopsis.info TAIR – http://arabidopsis.org Genevestigator – https://www.genevestigator.ethz.ch GABIpd - http://gabi.rzpd.de The Botany affymetrix database - http://bbc.botany.utoronto.ca:88

## 59 Deep profiling by massively parallel signature sequencing elucidates the small RNA component of the transcriptome

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Small RNAs play important regulatory roles in most eukaryotes but only a small proportion of these molecules have been identified. We adapted the method of massively parallel signature sequencing ("MPSS") to sequence more than two million small RNAs from seedlings and the inflorescence of the model plant Arabidopsis thaliana. These data increased the number of distinct small RNA sequences known by more than an order of magnitude. Most of the different sequences represent small-interfering RNAs (siRNAs) that match repetitive sequences, intergenic regions, and genes. Among the most abundant sequences were well-characterized microRNAs (miRNAs) and new miRNA candidates. Interestingly, no bias of small RNAs was observed towards genes with antisense transcription nor was there evidence that miRNAs cause transitivity, i.e. the production of siRNAs that match endogenous miRNA target mRNAs. In contrast, individual or clusters of highly-regulated small RNAs were readily observed. This powerful genome-wide method for identification and measurement of small RNAs extends miRNA prediction capabilities and is applicable to diverse organisms.

## 60 The Pyrabactins: small molecule agonists of the abscisic acid signaling pathway

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We performed a chemical genetic screen of a 10,000 member small molecule library to identify compounds that disrupt hypocotyl cell expansion in etiolated Arabidopsis seedlings. Six compounds were identified that inhibit germination and ~750 compounds were found to reproducibly inhibit hypocotyl growth by >20%. Two of the germination inhibitors identified are structurally similar and examination of an analog series shows that a pyridine moiety is essential for the activity of these compounds that we have named the Pyrabactins (for *pyr*idine *aba* a*ct*ivatio*n*).

To delineate their mechanism of action, the effects of the Pyrabactins were examined on mutants in the GA and ABA signaling and biosynthetic pathways. These experiments demonstrate that Pyrabactins require a functional ABA signaling, but not biosynthetic, pathway to inhibit germination suggesting that Pyrabactins activate the ABA signaling pathway. To examine this hypothesis further, whole genome transcript profiling was used and these experiments reveal that ABA and Pyrabactin treatments induce strikingly similar expression profiles (Pearson correlation coefficient of 0.89) Collectively our genetic, physiological and transcriptional data suggest that the Pyrabactins inhibit germination by activating the ABA signaling pathway and thus define a new class of synthetic plant growth regulators.

As a first step towards target identification two genetic approaches have been initiated. We have identified mutants resistant to Pyrabactin A, the strongest analog in the series, and these are under investigation. In addition, a screen of 120 ecotypes has uncovered several Arabidopsis isolates that are hypersensitive to the Pyrabactins; genetic analysis of one strain shows that Pyrabactin hypersensitivity segregates as a Mendelian trait that maps to the bottom arm of chromosome 3.

Our discovery of the Pyrabactins illustrates the successful application of forward chemical genetics in identifying new plant growth regulators and illustrates the utility of natural variation for identifying genetic factors that mediate the action of small molecules. Furthermore, the Pyrabactins should be useful tools for dissection and manipulation of the ABA signaling pathway.