Meeting report: 2013 FASEB Summer Research Conferences: Mechanisms in Plant Development.

August 11-16, 2013, Saxtons River, Vermont.

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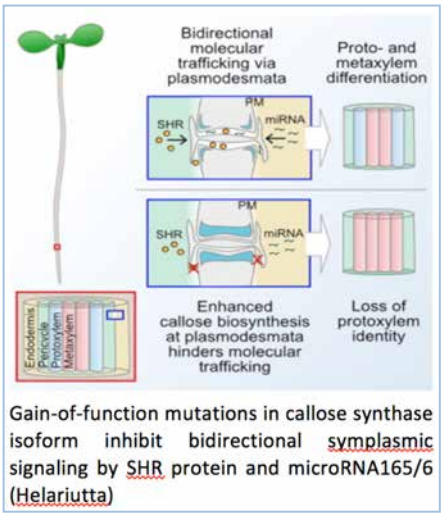
The 13th FASEB Plant Biology Conference was held from August 11- 16, 2013, in Saxtons River, Vermont, a modest but beautiful setting. This was a special meeting, since it marked 25 years since the first FASEB Plant Molecular Biology conference- the theme changed to Plant Development in the mid '90's. Around 160 attendees spent 5 days hearing the latest "developments" in plant development, and enjoying casual discussions in the swimming pond and the bar.

The meeting kicked off with an inspiring plenary talk by Prof. Liam Dolan (Oxford). Liam's lab has been studying root hair patterning and other aspects of root development in *Arabidopsis* for many years, but in this talk he described new work that seeks to understand the evolutionary origin of root- like structures in plants. Using a recently adopted liverwort model, Marchantia, which makes unicellular rhizoids, they performed a T-DNA screen for mutants affected in rhizoid development. One of them, called *rhizoidless*, was in a homolog of one of the *RSL* genes, previously characterized in their lab for a role in controlling root hair growth. Liam also described experiments to understand how stability of RSL proteins contributes to controlling root hair length, and also how RSL overexpression in crop plants might enhance biomass by improving nutrient uptake. It was inspiring to see such a broad presentation, from adopting new model systems, to potential agricultural gains that could result from this fundamental research.

Session 1. Local signals

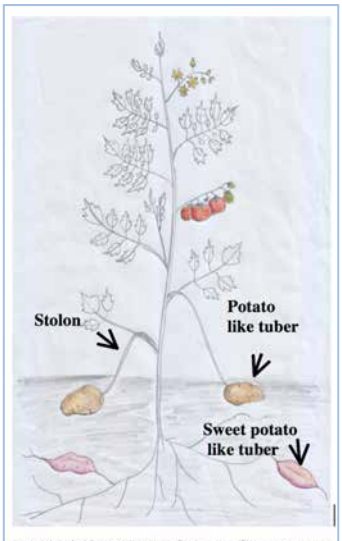
Anna Magdalena Pier (Max Planck Institute for Plant Breeding Research) and Margaret Frank (Cornell University).

In the opening session of the conference, the latest research on local signals was presented. The speakers showed their work on plasmodesmata-dependent miRNA movement, endosome-dependent protein movement and recognition of Calcium signals in roots, sensing of CO_2 concentration in leaves and cytokinin levels in secondary meristems. Furthermore, recent findings on the control of *Arabidopsis* embryo maturation and maize meristem size homeostasis were shared with the community.



Gain-of-function mutations in callose synthase isoform inhibit bidirectional symplasmic signaling by SHR protein and microRNA165/6 (Helariutta)

Ykä Helariutta and coworkers established an elegant system to study the impact of plasmodesmatal trafficking on root patterning in a cell type specific manner. They used cell specific promoters to drive the expression of an inducible Gain of Function allele of Callose Synthase 3 (icals3m) that blocks plasmodesmatal openings. Blocking symplastic trafficking in sieve elements resulted in a loss of companion cells, suggesting that a mobile signal originating from the sieve cells is essential for patterning the phloem. Previous work on phloem patterning revealed that the SHR-SCR-MIR165/6 pathway restricts the HD-ZIP III domain into the central region. Findings of Helariutta and coworkers indicate that movement of a protein from sieve elements to the surrounding phloem cells is required for coordinated asymmetric cell division, which is a crucial step in companion cell specification. Kimberly Gallagher proposed a signaling endosome model to explain the trafficking of the GRAS transcription factor SHORT ROOT (SHR), a key player in patterning of the root endodermis and cortex. The Gallagher lab showed that movement of SHR requires endosomal localization and its direct binding to SHR Interacting Embryonic Lethal (SIEL). They propose that endosomes serve as platforms for the regulation of SHR movement.

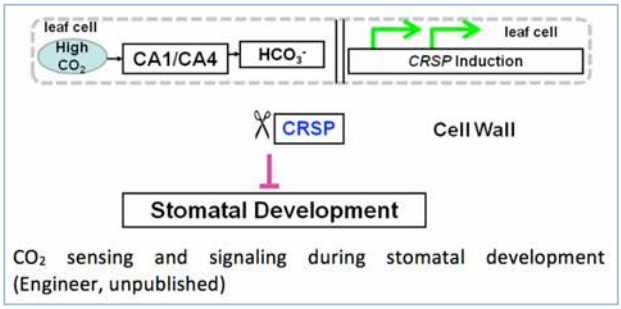


Potential of combining features from tomato and potato in a single plant (Eshed).

Yuval Eshed highlighted the morphogenic capacity of plants, using elevation of Cytokinin levels in tomato by overexpression of LOG1. LOG1 OX plants gain the potential to form swollen tissue structures from juvenile lateral buds, which accumulate starch and display expression profiles that are similar to potato tuber profiles. Like in potato, the potential to form such storage organs can be expended to all distal buds by overexpression of miR156.

Next, Giles Oldroyd presented the work of his group on the perception of Ca^{2+} signals for establishment of plant microbial associations. The recognition takes place via CCaMK, which is a calcium- and calmodulin-dependent

protein kinase that can bind Calcium at its CaM binding-and EF hand domains. Calcium binding of the EF hand domains primes the protein for inactivation, which takes place by autophosphorylation after Calcium binding of the CaM binding domain.



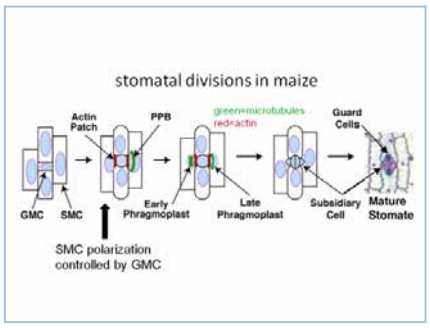
CO_2 sensing and signaling during stomatal development (Engineer, unpublished)

Cawas Engineer presented evidence for the requirement of a Carbonic anhydrase to suppress stomata formation in response to elevated CO_2 levels. The enzyme induces expression of the secreted protease CRSP, which functions in suppression of stomatal development. Pablo Jenik then presented his latest results on understanding how microRNAs and the transcription factors ASIL1 and ASIL2 prevent early embryo maturation in Arabidopsis, and what is known about the ASIL class of proteins. To wrap up, Michael Pautler discovered that the *fea4* mutant of maize encodes a bZIP transcription factor that defines a meristem peripheral domain, and activates a set of auxin response- and leaf differentiation genes. Remarkably, *fea4* mutants have greatly enlarged shoot meristems, whereas the Arabidopsis ortholog, *perianthia*, was previously characterized as having an increase in floral organ number without affecting meristem size.

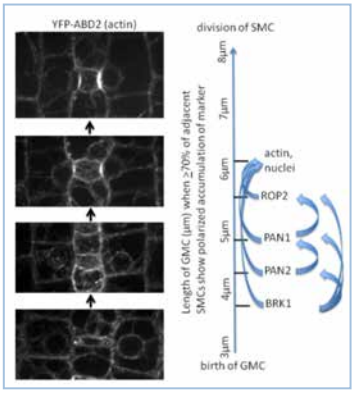
Session 2. Polarity.

Xian Qu (Cornell University) and Katie Abley (The John Innes Centre).

In the polarity session, Jeff Long presented new work on the genetic specification and maintenance of apical-basal polarity in the Arabidopsis embryo. Previous studies have shown that the transcriptional co-repressor *TOPLESS* plays an important role in maintaining embryonic apical-basal polarity. He then presented work ongoing in Marty Yanofsky's laboratory indicating the existence of a TPL-independent root specification pathway. Recently, Brian Crawford in the Yanofsky lab has found that a gene that regulates transmitting tract patterning in the gynoecium, and two of its close family members, help to pattern the embryonic root. Triple mutant embryos fail to initiate root formation, suggesting a previously uncovered developmental program involved in the specification of basal polarity. Mis-expression of one of these genes from the AS1 promoter causes ectopic roots to form on the tips of cotyledons. *In situ* hybridization data shows that the expression of these genes is normal in *tpl-1* mutant embryos, indicating that *TPL* does not control their expression in the apical half of the embryo and that they may function in a novel root specification pathway.

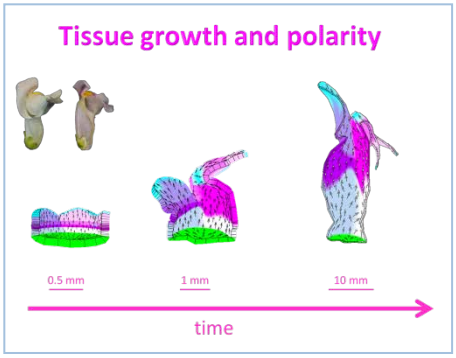


Also in the polarity session, Laurie Smith (UCSD) presented insights into the regulation of asymmetric cell divisions. During stomatal development in maize, guard mother cells (GMCs) guide the asymmetrical division of adjacent subsidary mother cells (SMCs). Laurie Smith presented recent work on how the physical cellular asymmetry, which precedes asymmetric division of SMCs, is established.



Previously, two leucine-rich repeat receptors PAN1 and PAN2 were found to function cooperatively to promote the polarization of SMCs, potentially by recognizing polarizing cues from the adjacent GMC. PAN2 acts upstream of PAN1, and PAN1 interacts with Type I ROP GTPases to polarize SMCs. More recently, the roles of BRK1 and BRK3 have been studied. BRK1 and BRK3 encode subunits of the SCAR complex, which promotes actin nucleation via activation of the ARP2/3 complex. Recent work shows that these SCAR complex subunits are required for the polarization of PAN1 and PAN2 in SMCs, implying that ARP2/3 complex dependent actin polymerization guides PAN1 and PAN2 polarization.

In the next talk, Remko Offringa described how regulation of the protein kinase PINOID (PID) can influence the pattern of PIN polarity within a tissue. PIN protein localization in the cell is dependent on the phosphorylation state of its central hydrophilic loop, and PID has previously been shown to phosphorylate PIN proteins at the central Serine in three conserved TPRXS(N/S) motifs, leading to their sorting in GNOM-independent trafficking pathways (apical/shootward, outer, indentation). Remko presented new data revealing how regulation of PID localisation by upstream regulators may allow environmental stimuli and endogenous cues to influence PIN localisation.



In a subsequent talk, Xana Rebocho from the Coen lab described the importance of the regulation of tissue polarity, and growth rates, for the morphogenesis of the Antirrhinum flower. During the development of the Antirrhinum flower, the ventral petal grows to acquire a folded shape which is necessary for the flower's closed mouth. Previous computer modelling studies gave rise to the predictions that the ventral petal primordium has a narrow horizontal ring of gene expression, which locally promotes growth and where, at intermediate stages of petal development, a polarity reorientation occurs to redirect principle orientations of growth. Dr. Rebocho presented work illustrating that expression of the CUP transcription factor, which is usually associated with boundary domains, correlates with the pattern predicted for the hypothesised growth promoting factor. *cup* mutants were shown to have a loss of ventral petal folding. Immuno-localisation of AmPIN1a revealed that, as predicted by the model, a reorientation of PIN1 polarity occurs during ventral petal development. This PIN1 polarity reorientation is lost in the *cup* mutant. This work suggests that the evolution of the closed mouth of Antirrhinum flowers involved modulation of the growth rates and polarity within the ventral petal caused by an alteration of the ancestral *CUP* expression pattern.

Session 3. Boundaries

Josh Strable (Iowa State University) and Sam Leiboff (Cornell University).

Shoot architecture of higher plants is determined by developmental events occurring at the growing tips. Meristems are the site of organogenesis, giving rise to lateral organs, such as leaves and axillary branches, which themselves have meristematic properties. A meristem contributes immobile cells to the formation of lateral organ primordia, and it is self-renewing. A major outstanding question is precisely how cells dynamically accept cell fate at the meristem-incipient organ boundary during organ initiation. An enthralling session devoted to boundaries featured studies that honed in on the genetic and molecular dissection of boundary specification and formation.

During shoot development, cells are fated accurately in the meristem-to-organ boundary, in part, through the action of the phytohormone auxin and ASYMETRIC LEAVES1 (AS1)/AS2 proteins in the incipient primordium, and the antagonistic action of KNOX proteins in meristematic cells. Rüdiger Simon (Heinrich-Heine University, Germany) presented compelling evidence that *JAGGED LATERAL ORGANS* (*JLO*), a *LATERAL ORGAN BOUNDARY DOMAIN* (*LBD*) gene family member, has an essential function in regulating cell fate in the meristem-primordium boundary in *Arabidopsis*. JLO functions in the boundary region to restrict *KNOX* expression, as *jlo* loss-of-function alleles show ectopic expression of *SHOOT MERISTEMLESS* (*STM*) and *BREVIPEDICELLUS* (*BP*). Furthermore, he showed that JLO could act as a homomeric complex and in heteromeric complexes with AS2 and AS1 proteins to repress *BP* expression in incipient lateral organs. Importantly, the JLO-AS2-AS1 complex was found to regulate *PINFORMED* (*PIN*) expression, auxin transport and auxin signalling components. Building on these data, Simon proposed that in *Arabidopsis* combinations of JLO and AS2 protein complexes orchestrate auxin dependent developmental processes through regulating *KNOX* and *PIN* expression during organ initiation.

The discussion on boundary dynamics continued with Marcus Heisler (European Molecular Biology Laboratory, Germany). He presented on cellular and molecular mechanisms in *Arabidopsis* that position incipient lateral organs and the establishment of adaxial-abaxial boundaries during their initiation. Auxin and the distribution of its efflux carrier, PIN1, create intracellular polarities that set up where a lateral organ will develop. As lateral organs arise in the peripheral domain of the shoot apical meristem (SAM), they adopt the pattern present in the radial axis of the SAM central zone. Organ polarity genes that are expressed centrally, like the Class III HD-ZIP gene family member *REVOLUTA* (*REV*), are expressed on the adaxial side of developing organs, whereas genes that are expressed more peripherally, such as *KANADIs* and *microRNA165/166* (*miR165/166*), are expressed on the abaxial side. Heisler reported findings that used an impressive coupling of live-image scanning confocal microscopy with an array of GFP-labeled molecular markers to decipher how central-peripheral/adaxial-abaxial patterning and cell polarity systems interact. During organ initiation, the boundary of *REV* expression extends from the SAM central domain to the outer peripheral domain where *KAN* and *miR165/166* are expressed. Auxin is required for extending *REV* expression, but its polar transport is not required for boundary position. However disrupting boundary position through ectopic expression of miR165/166 or a miR165/166 resistant version of *REV* resulted in ectopic organ formation centrally or organ suppression, respectively, suggesting that REV and

other Class III HD-ZIPs help define the auxin responsive organogenic zone. This concerted cross regulation allows lateral organs to emerge from a meristem precursor domain as adaxially-abaxially pre-patterned where at the cellular level, along an anisotropic growth axis, local auxin concentrations and auxin response cell-type patterning are tweaked further.

Leaf shape and axillary branching are conspicuous shoot architecture traits, which long have been considered regulated by unrelated developmental mechanisms. Klaus Theres (Max Plank Institute for Plant Breeding Research, Germany) reported in tomato a conserved mechanism regulates leaf complexity and axillary branching by controlling the morphogenetic competence of boundary cells during leaflet and axillary meristem imitation. He showed the paralogous MYB genes *Potato leaf* (*C*) and *Blind* (*Bl*), key regulators of leaf dissection and axillary branching, respectively, are co-orthologs of *Arabidopsis REGULATOR OF AXILLARY MERISTEMS* (*RAX1*) that have undergone sub- or neofunctionalization in tomato due to accumulated differences in their promoters. Theres also showed that *Goblet* (*Gob*) and *Lateral suppressor* (*Ls*) function in boundary regions to combine the regulation of leaf formation and axillary meristem initiation. Thus, the boundary genes *C*, *Bl*, *Gob*, and *Ls* may function to inhibit cell differentiation along the leaf margin and in the leaf axil.

The obvious conclusion from this engaging session is that biology of boundary region of higher plants continues to provide critical insights into the mechanisms that govern lateral organ formation. One future challenge is to continue to understand the importance of the proposed mechanisms *in vivo*. Another is to extrapolate our understanding of cell fate at boundary regions to other plant species with diverse shoot architectures.

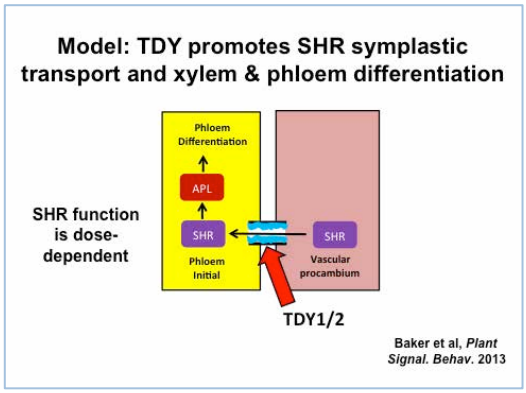
Session 4. Long range signals

Narender Kumar (Louisiana State University) and Katharina Schiessl (The John Innes Centre).

In the first talk of this session, Leslie Sieburth (Utah) presented new findings on the bypass signal. Proper development of shoot growth is the consequence of the interaction between shoot and root. A long-range signal is needed to coordinate their development. The Arabidopsis *bps1* mutant shows shoot and root arrest. Shoot arrest occurs because the mutant root makes too much of a substance, named the bps signal. This compound seems to be negatively regulated by *BPS1* because removal of *bps1* mutant roots restores shoot growth and wild type shoot growth arrests upon grafting of *bps1* mutant root. Using a micro-graft transient assay, the Sieburth lab has shown that *bps1* root signal is sufficient to repress *WUS* expression in wild type shoot apical meristem, and ectopic expression of WUS restores shoot growth.

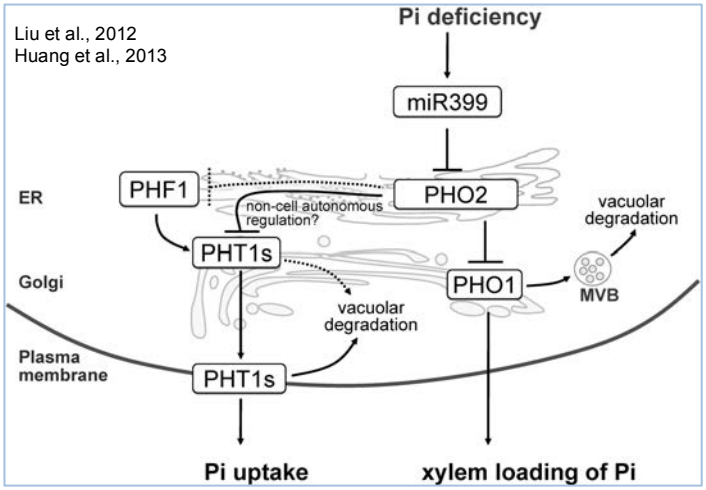
The next talk, by David Braun (Missouri) discussed two *Tie-Dyed* (*TDY*) genes, *TDY1* and *TDY2* in maize. Mutation in either gene gives the same phenotype, hyper accumulation of starch in the yellow regions of mutant leaves. *TDY1* is a recessive, null, developmentally stable mutation. The gene is expressed in phloem cells, the protein is localized in endoplasmic reticulum, and it contains a transmembrane domain, whereas *TDY2* is broadly expressed in developing veins and is predicted to encode a callose synthase. Callose is present in plasmodesmata and sieve plates. Mutations in *TDY2* or an

Arabidopsis callose synthase gene display xylem differentiation defects, although the *TDY2* mutation does not affect callose levels. Additionally, *tdy1* and *tdy2* mutant companion cells contain oil droplets. On the basis of the available evidence, it seems that the TDY proteins regulate symplastic trafficking of sucrose in the maize leaf.



In the next talk, Tzyy-Jen Chiou, Academia Sinica, Taiwan, presented her research on Regulation of Phosphate Uptake and Translocation by Long-Distance Signaling. The macro-nutrient phosphorus (P) is essential and rate-limiting for plant growth and development. To maintain homeostasis of inorganic phosphorus (phosphate, Pi) throughout the plant, the demand for Pi generated by processes in the shoot needs to be coordinated with the nutrient uptake from the soil in the root. Work by Chiou and co-workers identified the microRNA *miR399* as a systemic signal to communicate Pi availability and demand in the shoot to the site of Pi uptake in the rhizosphere. Grafting experiments provided evidence that *miR399* is expressed in the shoot first upon low Pi availability and systemically moves to the root where it targets the ubiquitin-conjugating E2 enzyme UBC24/PHO2. Quantitative membrane proteomics and a *pho2* suppressor screen together with co-localisation experiments provided evidence that PHO2 is localized at endo-membranes where it regulates the Pi uptake and root-to-shoot Pi translocations through mediating the protein degradation of the Pi transporter PHT1 and PHO1, respectively.

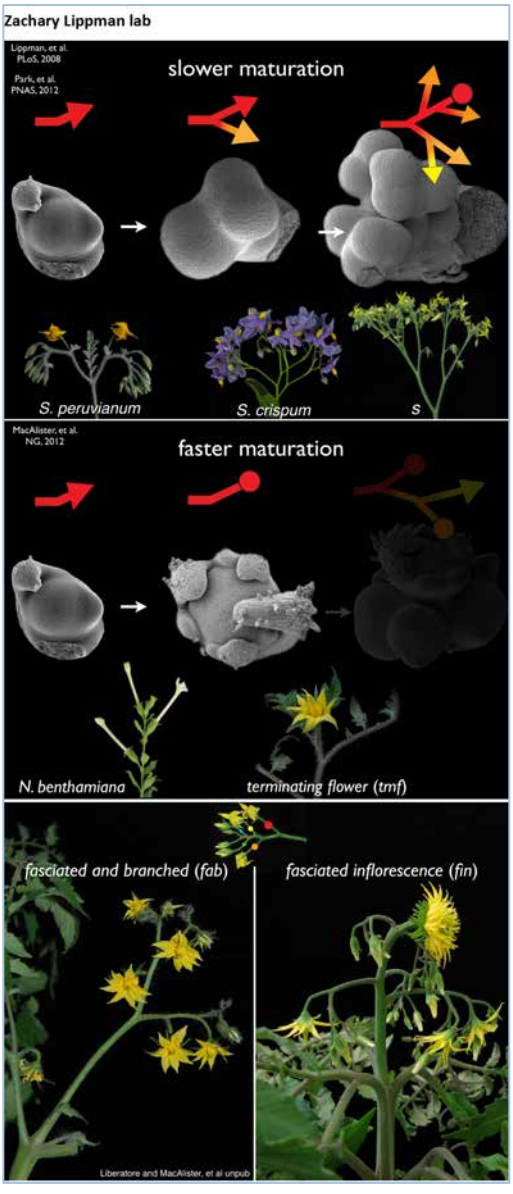
Accordingly, reduced Pi availability in the shoot of the *pho1* loss of function mutant caused up-regulation of *miR399* and a decreased in PHO2 activity. Conversely, high Pi- accumulation has been observed upon ectopic expression of *miR399* and in the *pho2* mutant, independently. Furthermore, in the shoot of the *pho1 pho2* double mutant very low level of Pi were detected in the shoot providing further evidence that PHO1 functions in the systemic translocation of Pi and acts downstream of PHO2. In summary, this work provides a mechanism how plants have adapted to maintain Pi homeostasis under low Pi availability, where *miR399* is up-regulated in the shoot, systemically moves to the root and targets the ubiquitin-conjugating E2 enzyme *UBC24/PHO2*. As a result, the proteins involved in Pi acquisition and transport such as PHT1 and PHO1 are stabilised and Pi uptake and systemic long distance transport of Pi are promoted.



In the last talk of the session, Catherine Rameau, INRA Versailles, discussed how Strigolactones and other long-range signals regulate shoot branching in pea. Strigolactone (SL) is a carotenoid derived phytohormone, which has diverse roles in plant development. SL mutant plants have been first described as high branching mutants. More recently, they have been shown to display more lateral roots, and less secondary growth. SL also regulates plant extension in moss. In her talk, Dr Rameau showed a summary of the pea branching model including the SL-receptor, the RMS3 protein very likely moving in the phloem sap. Using grafting and physiological characterization of the pea *rms* mutants, a novel shoot-to-root long distance signal RMS2-dependent has been identified. This signal regulates SL-biosynthesis and cytokinin export from roots in the xylem sap. Her lab recently cloned the *RMS2* gene, which encodes the pea homolog of the auxin receptor AFB4/5 from Arabidopsis suggesting that the long distance signal is auxin.

Session 5. Switches

Jeongmin Choi (University of Missouri) and Josh Strable (Iowa State University).

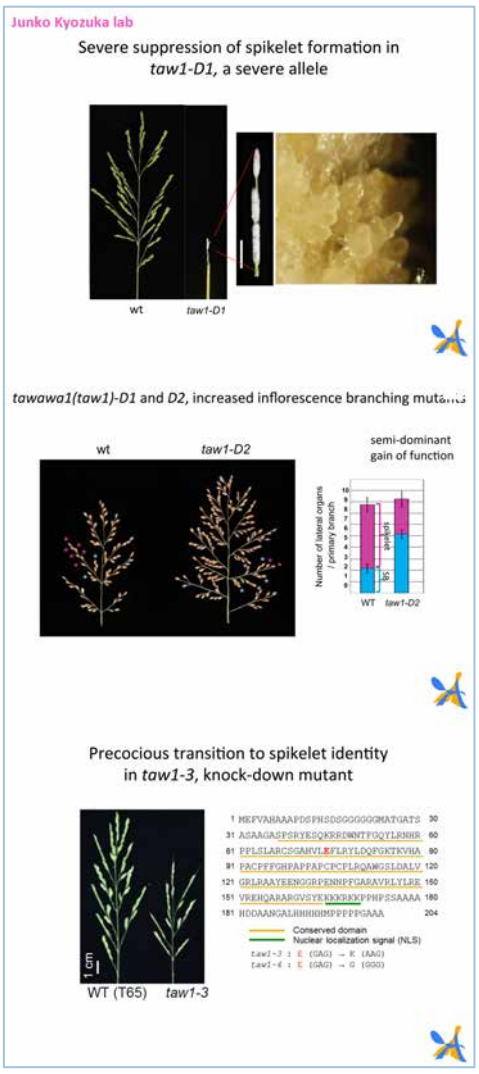


Compared to Arabidopsis, many crops exhibit unique inflorescence structures requiring more coordinated transition from stem cell maintenance to floral organ formation. Inflorescence architecture is largely determined by the fate of meristems, population of stem cells under specific genetic control. In the session on switches, novel molecular regulators of this inflorescence development were presented in three major crops: tomato, rice, and corn. In the first talk of this session, Zachary Lippman (CSHL) presented his labs' work on "Molecular switches, from stem cell maintenance to floral organ formation".

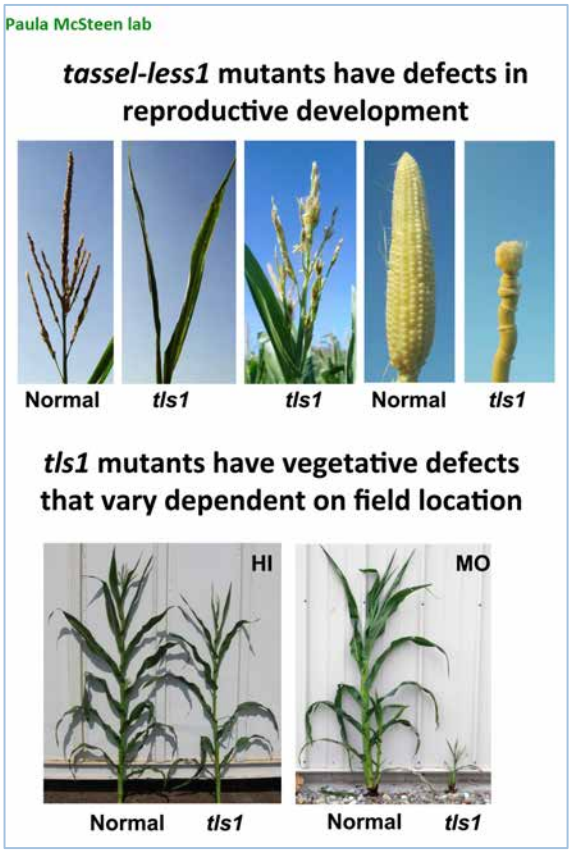
The diverse branching patterns of inflorescences in the nightshade (Solanaceae) family make it an ideal model for understanding the mechanism governing the transition from meristem to floral organ formation. Dr. Lippman introduced two tomato mutants, *fasciated and branched* (*fab*) and *fasciated inflorescence* (*fin*). Each mutant has an enlarged vegetative meristem, which upon floral transition, develops into more branched inflorescences with larger flowers and fruits. FAB and FIN encode an Arabidopsis CLAVATA1 ortholog and a plasma membrane localized protein of unknown function, respectively. The gain-of-function

mutation of *fab* confirmed that classical CLV-WUS pathway is also conserved in the tomato inflorescence meristem fate. Although the detailed mechanism of the *fin* mutant is still under investigation, much attention was to this mutant. In Arabidopsis, neither *fin-like 1*(*finl1)* and *finl2* single mutant nor double mutants displayed any phenotype similar shown to the tomato mutant. However, FINL1 and FINL2 function redundantly in pollen tube guidance, as the *finl1;finl2* double mutants set dramatically reduced seeds. The search for interacting proteins lead to a candidate, a glutamate receptor, which showed the similar pollen tube development defects in Arabidopsis. Considering that glutamate receptors allows cytoplasmic calcium flow, it is very intriguing to speculate whether calcium is involved in inflorescence meristem identity maintenance through the action of this novel protein, FIN, and its putative interacting partner, a glutamate receptor.

As in the case of tomato, regulating meristem identity greatly influences inflorescence architecture in rice. Junko Kyozuka (University of Tokyo, Japan) introduced a key regulator of the spikelet meristem to floral meristem transition, *TAWAWA1* (*TAW1*). The strong, dominant, gain-of-function allele, *taw1-D1,* produced highly branched compact inflorescences due to the severe suppression of spikelet formation. A weaker, dominant, gain-of-function mutant, *taw1-D2*, delayed spikelet formation resulting in more extensive branch development. In contrast, the *taw1* knockout mutant formed a smaller inflorescence due to the earlier transition. TAW1 encodes a nuclear protein of unknown function and belongs to the Arabidopsis, LSH1 and Oryza G1 (ALOG) gene family. In wild type plants, TAW1 is expressed strongly in branch meristems and disappears as a spikelet develops. TAW1 is also expressed in the vegetative meristem and the loss-of-function mutant produces fewer leaves.



In addition to genetic regulation of meristem fate, molecules, such as hormones and minerals also control meristem function. Lippman alluded to the critical role of calcium during inflorescence development in tomato. Similarly, the role of micronutrient, boron (B), as a potent regulator of corn meristem development was the focus of the talk by Paula McSteen (University of Missouri, USA). *tassel-less1* (*tls1*) mutants showed severe inflorescence meristem defects resulting in absent or reduced tassels and aborted ball-shaped ears. TLS1 encodes an ortholog of the Arabidopsis boron influx transporter, NIP5;1, a member the aquaporin family. Using a heterologous Xenopus oocyte system, she showed the B influx activity of TLS1. In addition, the B level in developing *tls1* meristem was

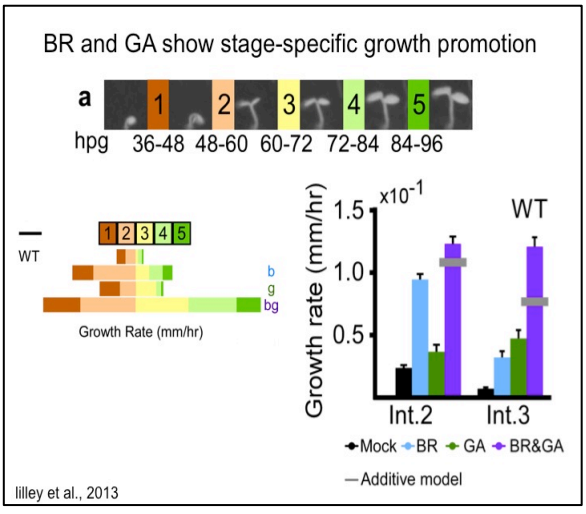


significantly lower and exogenous B recovered the meristem defects, suggesting the importance of B in meristem maintenance. Still the mode of action of how B plays a role in early stages of meristem development is not clear, however, several hypotheses were suggested. First, B might stabilize cell wall rigidity by cross-linking pectin. Second, it might directly affect the auxin homeostasis. From this talk, we learned that B has been supplemented in plant growth media for 90 years as an essential micronutrient perhaps due to an importance role in meristem function.

Inflorescence architecture impacts agricultural productivity. Signaling pathways controlling meristem size, the rate of development and the timing of the transition to floral organ initiation of these crops are directly related to our interest in improving crop yield. In the "SWITCHES" session, novel molecular and nutrient regulators governing this process were introduced; FAB, FIN, TAW1 and TASSEL-LESS1. In the next FASEB conference, we are expecting to learn more about how these regulators control the inflorescence development.

Session 6. Signal integration

Margaret Frank and Heather Meyer (Cornell University).

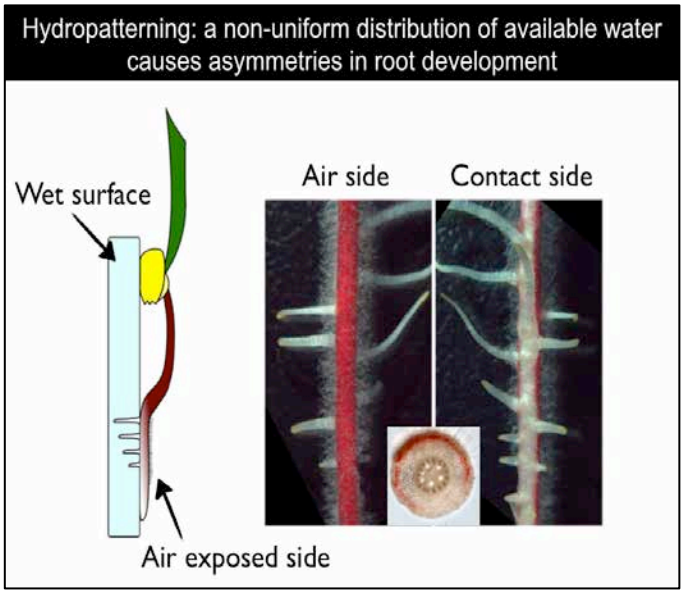


The signal integration session focused on the integration of molecular signals that coordinate complex environmental inputs and govern developmental outputs. The session highlighted an exciting array of developmental responses to the environment, from the phototracking of sunflowers to root patterning in heterogeneous soils.

Jennifer Nemhauser from the University of Washington started out the session by introducing the hypocotyl as a model system for measuring the role of complex environmental input on developmental output. The Nemhauser lab found that

when sucrose is supplied, hypocotyls keep growing as is they were in an earlier developmental stage. Nemhauser showed that this response to excess fixed carbon is likely a way to balance shoot and root growth to optimize plant metabolism. Interestingly, a parallel experiment looking at the influence of high CO_2 concentrations on shoot growth demonstrated that increased shoot biomass is abolished under low nitrogen conditions; suggesting that the seedling is growing to optimize both carbon and nitrogen assimilation. This seemingly simple hypocotyl system demonstrates the complexity of environmental inputs that govern growth tradeoffs. Nemhauser also presented data demonstrating the impact of developmental timing on crosstalk between the GA and BR pathways. She showed that GA and BR function additively in young seedlings to increase hypocotyl growth, but work synergistically in older seedlings. Strangely, growth repressors belonging to the DELLA family, which antagonize the GA pathway, are actually required for BR promotion of growth. These results highlight the importance of dynamic hormone signaling in pre- versus post- photosynthetic seedlings and more generally emphasize the role of developmental timing on hormone signaling.

In the next talk, José Dinneny of the Carnegie Institution for Science, Department of Plant Biology elucidated the role of heterogeneous water availability on lateral root patterning, a phenomenon referred to as "hydropatterning". The lab created an agar sandwich system where maize roots are grown between agar slabs with varying water potential. They found that lateral roots are preferentially initiated from the vascular pole closest to the wet agar surface and this initiation is independent of nutrient availability and mechanical touch. The Dinneny lab demonstrated that this asymmetric lateral root initiation is dependent on both auxin biosynthesis via the tryptophan amino transferase (TAA1) enzyme and polar auxin transport via PIN3. ABA treatment is sufficient to abolish hydropatterning and results in loss of PIN3 expression. How roots are sensing their local water availability remains a mystery but some possibilities include the sensing of water potential gradients in the soil and responding to oxygen availability.



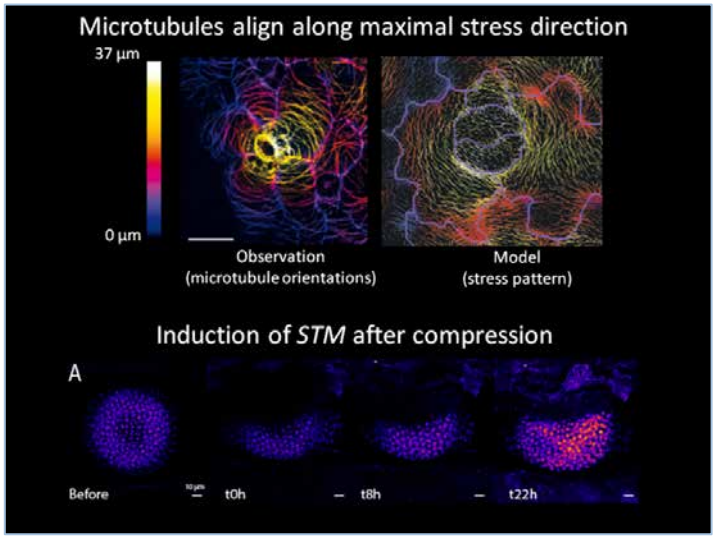
Stacey Harmer from UC Davis was the next speaker. She emphasized the impact of the circadian clock on transcriptomic patterns. The Harmer lab harvested tissue every 4 hours for 2 days and found that half of all genes are differentially expressed in a time dependent manner. When tissue is harvested at 30-minute intervals about 800 genes are differentially expressed and two thirds of these are clock related. Interestingly, expression of hormone regulated genes are significantly linked to the harvest time, indicating that hormone responses are regulated by the circadian clock. Harmer also introduced sunflower solar tracking as a model system to study circadian clock entrainment.

Sunflower heads track the movement of the sun from east to west and then return to face east after sunset in anticipation of dawn. The Harmer lab constructed a timed lighting setup that mimics the movement of the sun. They showed that sunflower repositioning towards the east is a circadian regulated movement; plants exposed to altered photoperiods lose the ability to reorient towards the east. In addition, phototropic sensitivity seems to be clock gated. When sunflowers were left under western light exposure rather than darkness at "night" they showed only a slight phototropic response at the beginning of the night and a strong phototropic response at the end of the night. The molecular mechanisms that regulate phototracking are not yet established, however, the lab discovered that the GA biosynthesis mutant, dwarf2, lacks the ability to phototrack and application of GA in a non-circadian dependent manner can recover phototracking. In addition, decapitation of floral buds leads to a loss of phototracking, suggesting that auxin may also play a role.

The final speaker for the session was José Alonso from North Carolina State University. Alonso presented on a very clever mutant screen that was performed to fish out genes coordinating the relationship between auxin signaling and ethylene response. Full-scale ethylene responses require wild type levels of auxin, suggesting there is an intrinsic link between the hormone signaling pathways. The Alonso lab identified two mutants that have both auxin and ethylene signaling defects: *ead1* and *ead2*. *EAD1* encodes a highly conserved PUA RNA binding domain and *EAD2* encodes a SUI1 RNA binding protein. Both EAD1 and EAD2 are associated with the 40s ribosomal subunit and are involved in mRNA translation. The Alonso lab used RNAseq and ribosome foot printing to show that ethylene induced changes are due to changes in both gene transcription and translation. Importantly, ethylene treatment lead to decreased translation of several proteins. These findings highlight a new level of regulation during auxin-ethylene crosstalk.

Session 7. Morphogenesis and shape

Heather Meyer (Cornell University), Katie Abley (The John Innes Centre) and Narender Kumar (Louisiana State University).

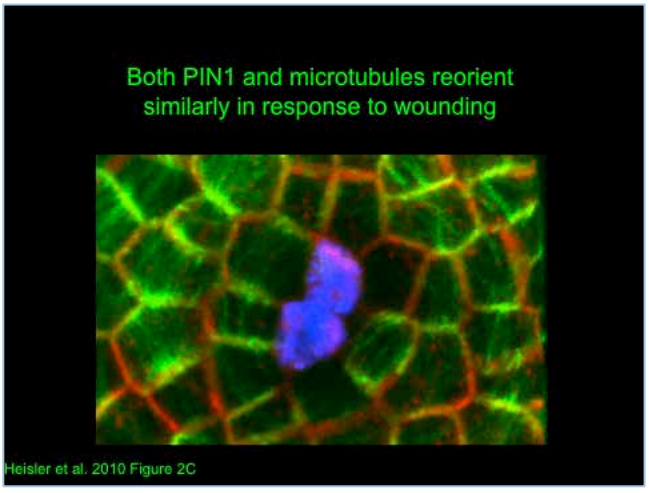


Olivier Hamant (Lyon) presented research on Mechanical signals in plant morphogenesis. It was recently proposed that a coupling between mechanical cues, microtubule (MT) alignment and PIN1 orientations plays a key role in morphogenesis and the generation of phyllotactic patterns. Hamant described a computational model that was used to generate predictions about the effects of MT-driven mechanical feedback on groups

of cells with local differences in target growth rates. It was predicted that weak feedback between mechanical stress and MT orientation tends to homogenise local growth rates, whereas stronger feedback causes more variability in the growth rates of neighbouring cells. In support of these predictions, WT meristems have considerable variability in the growth rates of neighbouring cells, suggesting relatively strong mechanical feedback, and in the *atktn1* mutant, which has a slower rate of MT reorientation, neighbouring cells have more homogeneous growth rates. New work suggesting that the feedback between mechanical stress patterns and microtubule orientations is not restricted to the shoot apical meristem was also presented. It was shown that local modulation of predicted stress patterns by the presence of raised stomatal guard cells in the leaf epidermis, and by ablation of pavement cells in the cotyledon epidermis, causes MTs in neighbouring cells to reorient and align along the predicted direction of maximal stress. A PhD student from the Hamant lab, Benoit Landrein, presented data showing that the expression of *STM* (which is enriched at the boundary) is elevated in response to mechanical perturbations such as meristem compression, suggesting that gene expression patterns, as well as microtubule orientations, are influenced by mechanical cues.

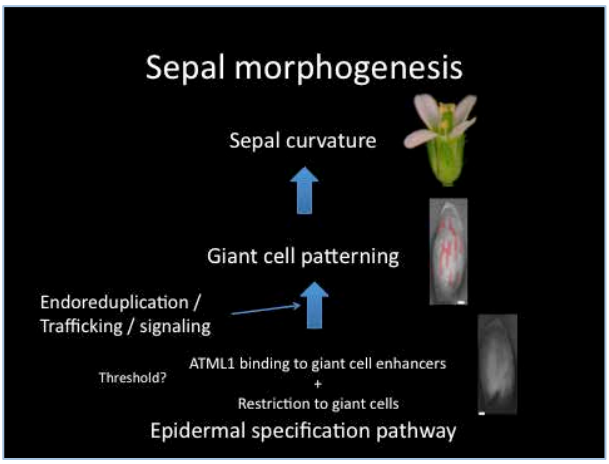
Uptal Nath focusses on the molecular basis of polar leaf growth. Leaf growth is often allometric, where leaves experience polar growth – more growth at the base and progressively less towards the tip. Utpal Nath's laboratory, from the Indian Institute of Science, has examined one-dimensional growth patterns along proximo-distal axis in 75 dicot species. They have discovered that the polarity of leaf growth can be diverse. The perennial species exhibit a diverse array of growth allometry (positive, with more growth at the proximal end than distal), mixed (more growth in the centre and progressively less towards either ends), and negative (more growth at the distal part than proximal). By contrast,all annual species studied exhibited only positive allometry. Cell proliferation and expansion always correlated with the direction of growth polarity. Additionally, Utpal's group investigated the relationship between the expression polarity of *miR396* and its target transcripts *GROWTH REGULATING FATORs (GRF)* - and leaf growth polarity. A strong correlation between growth polarity and expression of *miR396/GRFs* was observed, suggesting that expression diversity of miR396 underlies the diversity of leaf growth polartity.

Next, Elliot Meyerowitz (Caltech) presented work describing how physical and chemical signals control morphogenesis at the shoot apical meristem.



To study the morphogenesis of shoot apical meristem, we use genetic, genomic, cell biological imaging approaches that are relatively static. Prof Meyerowitz, in his talk, has suggested an approach in which all developmental biologists should consider the physical stresses acting in tissues and study morphogenesis as a whole using all parameters. Physical stress and the auxin

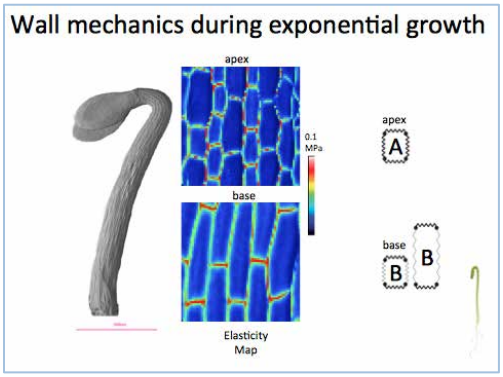
concentration pattern work in a feedback manner. Auxin can change physical properties of cell wall and cell wall stress can influence auxin efflux carrier in anisotropy in stressed cells. Stress patterns may dictate microtubule cytoskeleton, orientation, cellulose synthesis orientation in the cell wall, plane of cell division and the overall anisotropy of cells. Computational modeling can further help in understanding of these complex developmental processes. This approach and model has been successfully tested in his lab and those of his collaborators (Image taken by Marcus Heisler).



In the subsequent talk, Adrienne Roeder, Cornell University, discussed how coordination of cell division and cell type control the morphogenesis of the sepal.

The Roeder lab is using the Arabidopsis sepals as a model to study the morphogenesis of developing plant organs. Giant and small cells found in sepal have distinct molecular identities and their patterning is important in sepal morphogenesis. Loss of giant cells causes the sepals to curve inward and an increased number of giant cells cause the sepals to curve outward. The *ATML1*, *PDF2* and *ACR4* genes are important for shoot epidermal cell patterning and for the formation of giant cells in the sepal epidermis. *LGO* (encoding a cell cycle regulator involved in endoreplication), *SEC24A* (coding a COPII vesicle coat protein*)* and *GAI* (encoding a gibberellin signaling pathway protein*)* are involved in the formation of giant cells. Thus, the epidermal specification pathway, endoreplication and GA signaling may play a role in giant cell formation, which affects the sepal morphogenesis of Arabidopsis plant.

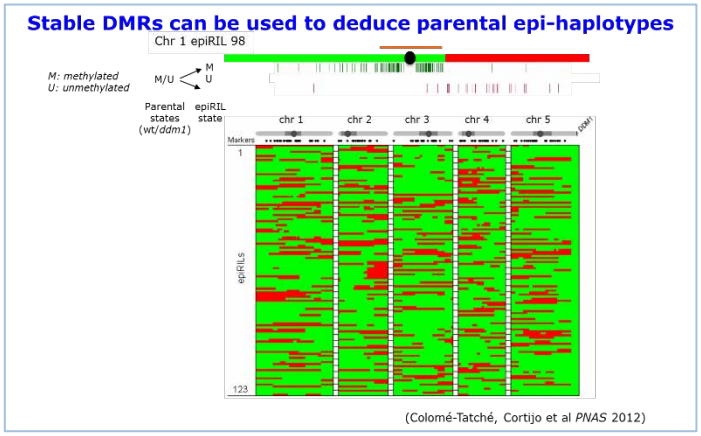
Siobhan Braybrook (Cambridge) next discussed how cell wall mechanics and pressure affect the overall shape of hypocotyl extension. To assess cell wall mechanics in hypocotyls, they used atomic force microscopy to measure elasticity of individual cell walls of plasmolyzed cells. Using this methodology, they observed that hypocotyl cells transition from having symmetric and isotropic growth to asymmetric and anisotropy growth during early development. More importantly, during cell elongation, they demonstrated that individual cells' walls exhibit differential cell wall elasticity. Siobhan has partially attributed differences in cell wall elasticity to pectins. In *echidna* mutants – which are deficient in pectin –hypocotyls exhibit a reduced etiolation phenotype. Furthermore, auxin transport was found to be important for hypocotyls elongation; vesicle trafficking inhibitors BFA and ConcA reduced etiolation**.**



Session 8. Evolution of Developmental Mechanisms

Sam Leiboff (Cornell University) and Anna Magdalena Pier (Max Planck Institute for Plant Breeding Research).

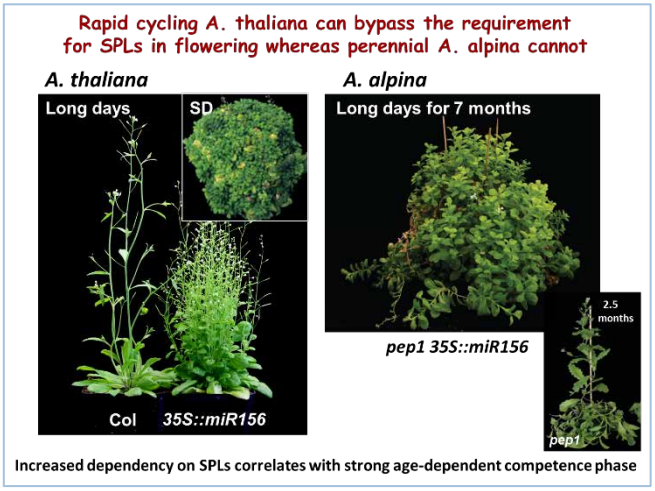
In the last session, participants returned to the Vermont Academy auditorium after an afternoon of outdoor activities. Refreshed by a game of volley ball, a jog through the woods, or a swim at the local pond, attendants were pleased to hear three fantastic stories outlining recent discoveries in developmental phenomena related to species evolution. These three presentations illustrated the power of mutant screens in carefully-chosen model systems to dissect specific molecular mechanisms of speciation in plants. Utilizing described mutants and/or novel mutant screens, these three groups were able to provide new findings on the potential for epigenetics to affect phenotypic variation, the mechanisms that regulate triploid infertility, and the regulation of annual and perennial life history in closely related species.



Vincent Colot of the Institut de Biologie de l'Ecole Normale Supérieure presented his group's recent work exploring the link between genome sequence, DNA methylation status, and plant phenotype. He began by outlining the creation of the *Arabidopsis thaliana* epiRILs, a population of plants designed to study epigenetic inheritance. By crossing a plant deficient in DNA methylation (with a lesion in *DDM1*, the SWI/SNF chromatin remodeler) with its wild-type counterpart, then backcrossing to restore competence in DNA methylation and selfing of individuals homozygous for the wild type *DDM1* allele, Colot's group was able to study the transmission of differentially methylated regions (DMRs) in the *Arabidopsis* genome across generations. They found that amongst the several thousand DMRs, most of which correspond to repeat elements, approximately 1 in 3 was inherited stably. Tracking DMRs between a few hundred and 25k base-pairs in length, they observed the gradual re-methylation of certain DMRs inherited from the DNA methylation-deficient parent. They demonstrated that this re-methylation is accomplished during reproduction by the RNA-directed DNA methylation pathway and have determined that the size of the repeat family to which the DMR belong as well as the degree of sequence identity of the DMR to other family members are correlated to re-methylation. Colot's group made use of the epiRILs to track the effect of de-methylation on previously repressed transposons. Interestingly, they have found that de-repression of transposable elements is not always linked to activation of transposition. Meanwhile, they have used the epiRILs to create the first genetic map ever built using DMRs as markers. They then used QTL analysis to identify DMRs that cause heritable variation for several quantitative traits, namely flowering time, plant height, and root length. Through these and other experiments they have been able to provide direct

evidence that epigenetic variation provides a rich source of heritable phenotypic variation.

Next, Claudia Köhler of the Linnean Center of Plant Biology, Uppsala, Sweden, presented her group's recent breakthroughs in understanding the mechanisms behind the 'triploid block' that causes the post-zygotic isolation of newly formed polyploid progeny. Normally, tetraploid endosperm that is generated from a diploid x tetraploid cross is unable to correctly cellularize during seed development, leading to embryo abortion. Through a novel mutant screen her group was able to identify suppressors of the triploid block in *Arabidopsis thaliana*. Köhler presented her group's characterization of a suppressor of the triploid block, a novel gene, whose function may participate in rapid speciation.



George Coupland of the Max-Planck-Institute for Plant Breeding Research, finished off the session by presenting his group's recent findings on the molecular mechanisms behind vernalization (induction by cold treatment) and age requirements for flowering in the genus *Arabis*. By comparing developmental pathways in perennial and annual species, Coupland's group has been able to identify molecular factors which contribute to drastically different life histories within this genus. Vernalization induces the perennial species, *Arabis alpina* to flower only if the plant has passed a prerequisite age. Coupland's group was able to use this system to dissect the effects on vernalization and age on flowering in this perennial. They found that vernalization is able to induce *LEAFY* (*LFY*), required for flowering, but can only do so in shoot apices that have surpassed the prerequisite age. This provides a mechanism for a perennial plant to convert some, but not all meristematic tissues to the production of flowers, reserving apices for continued vegetative growth and persistence in the environment across seasons. Through mutant screens of *A. alpina* looking for deficiencies in flowering inhibition Coupland's group was able to identify several *PERPETUAL FLOWERING (PEP)* genes, which function in the induction of flowering by vernalization, but were unable to abolish the age requirement for flowering. To examine the regulation of age-dependent competency for flowering, Coupland's group used a microarray experiment comparing the transcriptional profile of competent and non-competent plants. Interestingly, Coupland's group identified several *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes that are upregulated in competent plants. Knowing that *SPL* genes are targeted by miR156, Coupland's group performed several experiments beautifully illustrating the antagonism between miR156 and SPLs, showing that miR156 is a key repressor of flowering, required for age-dependent competence. However, Coupland's group found that *Arabis montbretiana,* a related annual species has lost this particular regulation mechanism and is no longer inhibited by miR156, allowing the adoption of an annual, rapid-flowering life history.

The conference ended with a thoughtful overview by Scott Poethig (U. Penn). He reminded us of how our field has come full circle, from a situation where everyone studied a different plant, through a bottleneck where almost everyone worked on Arabidopsis or one or two other model systems, to a new era where genomics allows us to again use diverse species to tackle important problems in plant development. He also reminded us how some concepts discussed at the meeting had been discussed much earlier, in particular reminding us of the seminal ideas of Paul Green in thinking about the role of mechanical forces in plant morphogenesis. Overall it was a truly memorable meeting, and we look forward to the next FASEB Mechanisms in Plant Development meeting in 2015, which will be organized by Dominique Bergmann and Rudiger Simon.