

Meeting report

## Moonlight in Vermont illuminates plant development

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This year's FASEB meeting on plant development covered a wide range of topics, including patterning, cell specification and the evolution of developmental mechanisms. The plant hormone auxin and some recently discovered small regulatory RNAs emerged clearly as key regulators of many developmental processes. Also notable was the variety of tools involved in the work presented, ranging from molecular genetics and genomics to imaging and computational modeling.

### A feast of auxin

Many presentations showed how highly specific patterns of auxin distribution strongly influence plant growth and development. Establishment and maintenance of such patterns depend largely on active transport mechanisms mediated by the PIN family of efflux carriers, which localize to the plasma membrane at one pole of the cell. Cellular responses to auxin involve rapid changes in gene expression regulated by auxin response factors (ARFs) and AUX/IAA proteins. ARFs bind directly to DNA to activate (or repress) transcription, a process inhibited by their heterodimerization with AUX/IAAs. Auxin derepresses ARF functions by promoting the ubiquitination and degradation of AUX/IAAs.

During embryogenesis, the zygote undergoes cell division, growth and cell-type specification events that ensure the correct positioning of embryonic organs. These early events establish the plant body plan by defining the shoot and root positions (called poles) and the upper (adaxial) and lower (abaxial) surfaces of leaves. The first *Arabidopsis* asymmetric zygotic division produces a small apical

cell, which generates the proembryo, and a larger basal cell, which forms the extraembryonic suspensor attaching the embryo to maternal tissues. The proembryo then recruits the uppermost suspensor cell to form the root meristem founder cell (the hypophysis). PIN-dependent polar auxin transport and activity of *ARF5* (also called *MONOPTEROS*, *MP*) and *IAA12* (also called *BODENLOS*, *BDL*) are all required for specification of the hypophysis. However, during the time of hypophysis specification, *MP* and *BDL* are expressed only in the apical cell derivatives, suggesting that they act non-cell-autonomously to specify the hypophysis (they are required in cells other than those in which they are produced).

Dolf Weijers (Wageningen University, Wageningen, The Netherlands) showed that *MP* and *BDL* do not themselves move but, rather, they generate downstream signals from proembryonic central cells to the immediately adjacent hypophysis. One mobile signal is auxin itself, because *BDL*-dependent *MP* activity induces *PIN1* expression and auxin translocation to the hypophysis. However, auxin is insufficient for hypophysis specification, a finding that prompted the search for additional target genes of *MP* that potentially act non-cell-autonomously. A promising candidate, *TARGET OF MONOPTEROS3* (*TOM3*), is indeed transcribed in the upper cells of the embryo, whereas its protein product, a putative AP2-domain transcription factor, accumulates in the hypophysis. Jeff Long (Salk Institute, La Jolla, USA) provided clues to how embryonic apical fates are specified, a process compromised at the transitional stage in the *tpl-1* dominant-negative allele of *TOPELESS* (*TPL*), which encodes a transcriptional co-repressor. In *tpl-1*, shoot poles are transformed into root poles, giving rise to double-rooted seedlings. This phenotype provided the basis for an ethyl methane sulfonate (*EMS*) suppressor screen, leading to the identification of *big top* (*bgt*, encoding a histone acetyltransferase) and *top heavy* (*tph*, a new allele of *PHABULOSA*,

*PHB*). Thus *PHB*, a class III homeodomain-leucine zipper (HD-ZIP) transcription factor required for adaxialization in post-embryonic tissues, might also specify shoot pole identity during embryogenesis. A yeast two-hybrid screen for TPL-interacting proteins identified transcription factors from different families - all containing the ERF-associated amphiphilic repression (EAR) domain - together with *AUX/IAA* proteins. Long suggested that TPL normally co-represses ARF-mediated transcription and that, in *tpl-1* mutants, repression remains unaffected by auxin because *tpl-1* suppresses the *bd1* phenotype.

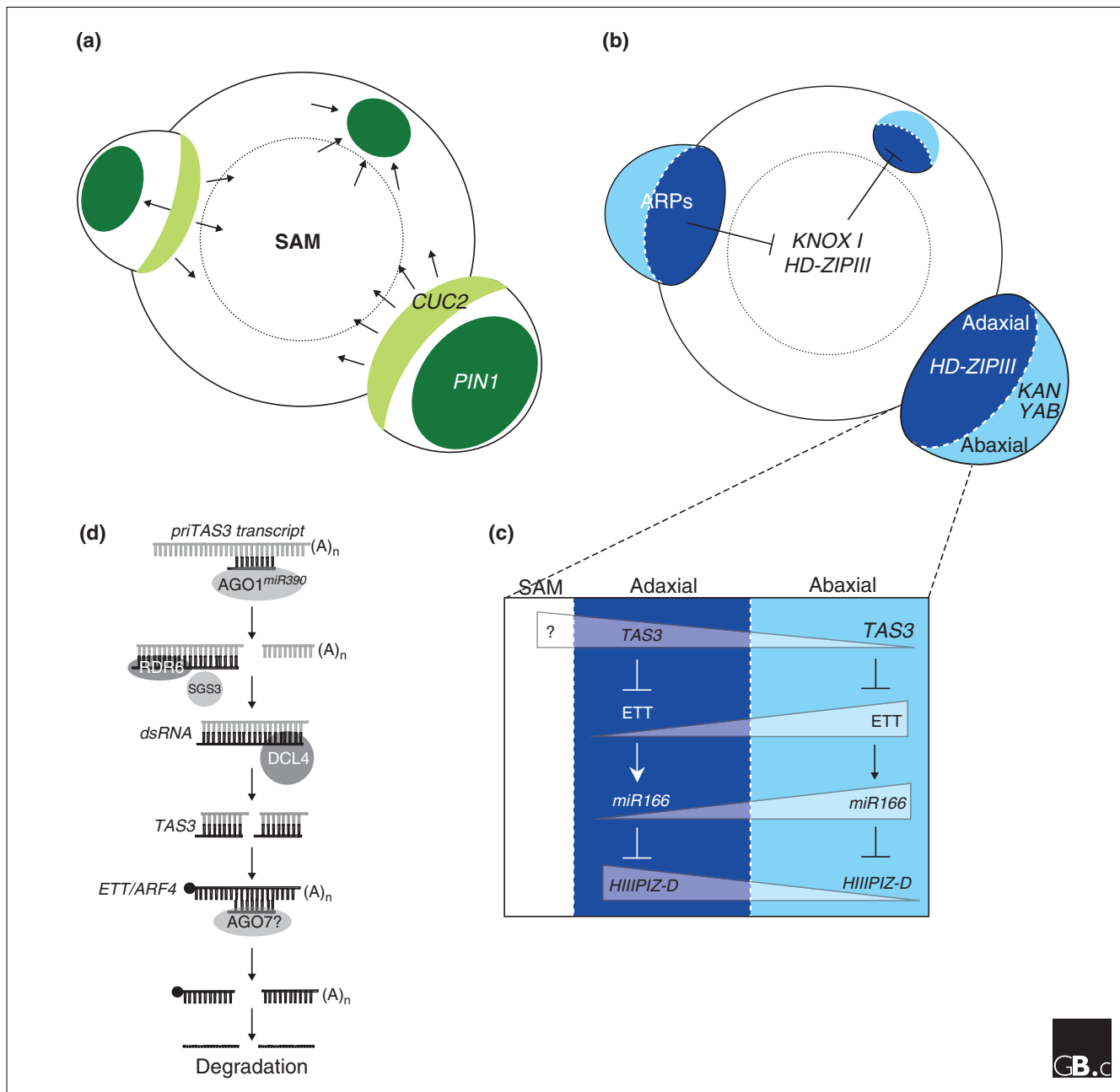
The *HD-ZIPIII* and *KANADI* (*KAN*) family of genes have complementary expression patterns and antagonistic activity in the specification of organ polarity (Figure 1b). John Bowman (Monash University, Melbourne, Australia) showed how both these classes of transcription factors can affect vascular differentiation and basic body-plan development through the control of auxin distribution. Ectopic organs and vascular bundles in *kan* mutants and lack of bilateral symmetry and shoot apical meristems (SAMs) in *hd-zipIII* mutants all correlated with altered PIN1 expression. Bowman proposed that the *KAN* genes negatively regulate auxin flow by regulating the *PIN* genes whereas the *HD-ZIPIII* genes are positively regulated by auxin. He also proposed that apical and vascular meristems share common patterning mechanisms involving *HD-ZIPIII* and *KAN* activities. The conservation of these genes in all land plants suggests that they have ancestral functions in establishing three-dimensional growth patterns, functions that preceded the evolution of leaf vascularization.

Many talks showed the importance of auxin in organogenesis and in specifying the regular arrangement of lateral organs around the stem (phyllotaxis). This process depends on the SAM, a group of undifferentiated cells producing organ primordia and new meristems at its flanks. Cris Kuhlemeier (Berne University, Berne, Switzerland), who presented work done in collaboration with Przemyslaw Pruswinkiewicz (Calgary University, Calgary, Canada) and Elliot Meyerowitz (Cal-Tech, Pasadena, USA), illustrated the benefits of mathematical modeling for understanding the role of auxin in patterning. The speakers presented related models that assume that patterning occurs in the SAM epidermal layer, where auxin polarizes its own efflux towards newly emerging primordia through the action of PIN1. In both models, PIN1 distribution in a given cell depends on the relative auxin concentration in neighboring cells. PIN1 preferentially polarizes towards adjacent cells with the highest auxin content, pumping even more auxin into these cells, thereby creating regular alternation of auxin-enriched and auxin-depleted areas (Figure 1a). When integrated into models of normally growing meristems, *Arabidopsis* phyllotaxis was convincingly reproduced. Meyerowitz's model could also predict rapid PIN1 polarization reversals during primordium development.

Despite these advances, important questions remain unapproachable by predictive models, for example, how cells sense auxin concentrations, how auxin influences PIN polarity and how it affects growth and patterning. Michael Sauer (Tübingen University, Tübingen, Germany) provided evidence that *AUX/IAA* pathways control the tissue-specific reorientation of PINs, and Meyerowitz showed how three-dimensional time-lapse imaging of inflorescence meristems could help understanding of growth and patterning. Cell divisions were monitored over time at the same time as the dynamic expression of several transcription factors was measured by labeling them with green fluorescent protein (GFP): the factors were ones required for meristem maintenance (*SHOOT MERISTEMLESS*, *STM*) and organ separation (*CUP-SHAPED COTYLEDON2*, *CUC2*) or for organ polarity (*REVOLUTA*, *REV*; *FILAMENTOUS FLOWER*). Combined with analyses of PIN1-GFP localization (which reports cell division and auxin distribution), the results suggested that auxin transport, besides determining the position of organ primordia, also regulates their subsequent differentiation by controlling expression of these genes. Interestingly, primordia can emerge where expression of adaxial fate-specifying factors (such as *REV*, which is auxin-dependent) and abaxial fate-specifying factors (such as *KAN*, which is auxin-independent) coincide, reminiscent of the overlap between abaxial and adaxial fates required for leaf blade expansion.

In plants with simple leaves (such as *Arabidopsis*), expression of *KNOX* transcription factors (including *BREVIPEDICELLUS*, *BP*) is confined to the meristem by the activity of actin-related proteins (ARPs) such as *ASYMMETRIC LEAVES1* (*AS1*; Figure 1b). In *Arabidopsis*, restricted expression of *AS1* in leaf primordia maintains the repression of *BP* and enables leaf initiation, coincident with auxin accumulation. Angela Hay (Oxford University, Oxford, UK) proposed that the *AS1* and auxin pathways converge to negatively regulate *BP*, because the *as1* mutant phenotype is enhanced in an auxin-insensitive background (*auxin-resistant1*): leaves had dramatically lobed margins and ectopic stipules as a result of ectopic *BP* expression. Hay also discussed how disrupted auxin gradients, resulting from *BP* misexpression, can alter leaf shape.

Miltos Tsiantis (Oxford University) showed that, in contrast to the exclusion of *KNOX*s from *Arabidopsis* leaves, their expression is required to delay differentiation and produce dissected leaves in the close relative *Cardamine hirsuta*. Differences in *KNOX* expression between *Arabidopsis* and *C. hirsuta* arose through changes in their regulatory sequences, even though *AS1* expression and function is conserved between the two species, indicating that evolutionary tinkering with *KNOX* regulation, constrained by ARP function, has produced diverse leaf forms. The existence of a developmental constant in the shoot-leaf transition involving *KNOX/ARP* proteins was evidenced by Jane Langdale



**Figure 1**

A model integrating the multiple layers of regulation that account for the development and polarization of leaf primordia from the shoot apical meristem (SAM) in plants. **(a)** The role of auxin effluxes, based on the models of Meyerowitz and Kuhlemeier. Top view of a shoot, showing the SAM (inside the dotted line) and three developing leaf primordia at different stages. Dark green, high levels of auxin; light green, low auxin levels; arrows, movement of auxin; PIN1 and CUC2 are expressed in the high and low auxin regions, respectively. In both models, the PIN1 distribution in a given cell depends on the relative auxin concentration in neighboring cells. PIN1 preferentially polarizes towards adjacent cells with the highest auxin content, pumping more auxin into these cells. **(b)** Regulation of gene expression by transcription factor proteins. Expression of ASYMMETRIC LEAVES1 (AS1) is restricted to the leaf primordia; this maintains the repression of KNOX proteins in the leaf primordia and enables leaf initiation when auxin accumulates. KNOXs, which specify meristem identity, are thus confined to the SAM. HD-ZIPIII and KANADI (KAN) genes have complementary expression patterns in leaf primordia; they act antagonistically to each other to specify the polarity of the emerging leaf. **(c)** A close-up of the leaf primordium in (b), showing opposing gradient of protein and small RNA expression. Cell-to-cell movement might cause a reduction in levels of TAS3 from the SAM vasculature to the abaxial side of the developing primordium. This might confine HD-ZIPIII expression to the adaxial domain. The model is based on the proposal by Timmermans that miR166 expression is transcriptionally controlled by ETT, which is itself a known target of TAS3. **(d)** The pathway of ta-siRNA biogenesis. First, a non-coding transcript is cleaved by the miRNA-loaded Argonaute1 (AGO1), the 'slicer' component of the RISC complex. Cleavage fragments are then converted into dsRNA by SGS3 and RDR6, from which ta-siRNAs are generated by the action of DCL4. The ta-siRNAs then cleave and degrade target transcripts bearing sequences complementary to the ta-siRNA, possibly through the recruitment of an AGO7-programmed RISC.

(Oxford University, UK), who showed these factors were recruited at least twice independently in land plant evolution (in lycophytes and euphyllophytes) to generate different types of leaves. Finally, Neelima Sinha (University of California, Davis, USA) used global gene expression profiling data and quantitative trait locus analyses to isolate additional factors regulating leaf complexity in *Solanum* species, also revealing a role for the polarity gene *PHANTASTICA* in such a process.

### Small regulatory RNAs

Small interfering RNAs (siRNAs) and microRNAs (miRNAs) have emerged as essential regulators of eukaryotic gene expression. In particular, miRNAs have been shown to be crucial in plant and animal development. In plants, the RNase III enzyme Dicer-like-1 (DCL1) processes miRNAs from imperfect stem-loop RNAs transcribed from intergenic or intronic regions. Upon incorporation into an RNA-induced silencing complex (RISC), plant miRNAs are believed to cleave cellular transcripts bearing miRNA-complementary sequences, but the underlying mechanism remains poorly understood.

One of us (O.V.) presented the results of a forward-genetic screen using a GFP-based sensor mRNA with a fully complementary miRNA-binding site at its 3' end and suggested that, in addition to specifying mRNA cleavage, plant miRNAs might also promote widespread translational inhibition and/or protein degradation. He also suggested that the second process specifically inactivated in certain mutants uncovered by this screen might occur in dedicated sub-cellular structures.

Leslie Sieburth (University of Utah, Salt Lake City, USA) presented a screen for *Arabidopsis* mutants with cotyledon and leaf vein patterning defects that led to identification of plant orthologs of two major constituents of P-Bodies, in which mRNA decay and miRNA-directed translational inhibition are known to occur in animals. VARICOSE (VCS) is homologous to a WD-domain protein that facilitates interactions between the human mRNA decapping enzymes hDCP2 and hDCP1; TRIDENT (TDT) is orthologous to hDCP2 itself. Sieburth showed that cellular target transcripts of all (or several) miRNAs accumulate normally in *ttd* and *vcs* mutants, although the miRNA target protein levels were not tested. TDT:GFP fusions form cytoplasmic speckles reminiscent of P-bodies.

In *Arabidopsis* leaves, abaxial expression of the miRNAs *miR165* and *miR166* directs degradation of the *PHB* and *PHAVOLUTA* (*PHV*) mRNAs, thereby restricting their accumulation to adaxial domains (Figure 1c). Marja Timmermans (Cold Spring Harbor Laboratory, New York, USA) showed that, similarly, *miR166* targets the *HD-ZIPIII* family member *rolled leaf1* (*rld1*) to specify

adaxia-abaxial polarity in maize leaves. Levels of *miR166* peak below incipient leaves and form a gradient, suggesting that *miR166* is spatiotemporally regulated by a mobile signal, or might itself be mobile (Figure 1c). Timmermans reported that *leafbladeless1* (*lbl1*) is required for proper *rld1* and *miR166* expression. The *lbl1* gene is orthologous to *Arabidopsis* *SUPPRESSOR OF GENE SILENCING3* (*SGS3*), required for synthesis of *trans*-acting (ta)-siRNAs, a newly discovered class of small RNAs. Biogenesis of ta-siRNAs involves miRNA-directed processing of primary transcripts, conversion of the resulting precursor RNAs to double-stranded RNAs through RNA-DEPENDENT RNA POLYMERASE6 (RDR6) and *SGS3* activities, and phased DICER-LIKE4 (DCL4)-mediated processing (Figure 1d).

Scott Poethig (University of Pennsylvania, Philadelphia, USA) reported that RDR6 and *SGS3* were originally identified through a genetic screen for *Arabidopsis* mutants with a compromised juvenile-adult phase transition. Transcript profiling and searches for suppressors of *rdr6* and *sgs3* converged in the identification of *ETTIN* (*ETT*, also called *ARF3*) and *ARF4*. Both transcription factors are targeted by *TAS3*, an evolutionarily conserved ta-siRNA whose accumulation requires *miR390*-directed cleavage (Figure 1d). Expression patterns and loss-of-function analyses suggest that *ETT* and *ARF4* regulate the sensitivity of juvenile-adult phase changes to a temporal, possibly mobile signal. Interestingly, Timmermans showed that loss of *lbl1* function reduces accumulation of *ta-siR2142*, the maize *TAS3* ortholog. Binding sites for *ARF3* and *ARF4* (both targeted by *ta-siR2142*) are found in the promoters of some of the several *miR166* genes in maize, and this could explain how reduced *ta-siR2142* levels cause misexpression of *miR166* in *lbl1* mutant leaves. Thus, *miR166* transcription might normally be inhibited in adaxial domains through *ta-siR2142*-mediated repression of *ETT* and *ARF4* (Figure 1c). Because *ta-siR2142* accumulation is itself dependent upon *miR390*, this example illustrates the complexity expected from small RNA-directed developmental mechanisms.

The ta-siRNA pathway thus regulates phase change and leaf polarity by controlling *ARF4* and *ETT*. Given that miRNAs target other ARFs and that ta-siRNA biogenesis requires miRNA-directed cleavage, why do miRNAs not directly regulate *ETT* and *ARF4*? The answer possibly lies in the fact that both Poethig's and Timmermans' models involve mobile signals. However, miRNAs act in a spatially restricted manner. By contrast, siRNAs exert their effects away from their sites of synthesis and amplification by DCL4 and RDR6, respectively. As endogenous RDR6 and DCL4 products, ta-siRNAs thus have all the required features to signal adaxial fates between cells (Figure 1c) and perhaps phase changes in whole organisms. It is in fact conceivable that ta-siRNA pathways could have evolved specifically to convey the regulatory effects of cell-autonomous miRNAs in distant tissues.

It is becoming ever clearer that multiple layers of regulation control patterning and organ growth in plants. 'Classical' protein-based developmental programs must now be integrated into the broader contexts of hormone signaling and small RNA-directed functions. One important challenge will be to address how those networks interact with each other and how redundant they are.