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Auxin at the Shoot Apical Meristem

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Plants continuously generate new tissues and organs through the activity of populations of undifferentiated stem cells, called meristems. Here, we discuss the so-called shoot apical meristem (SAM), which generates all the aerial parts of the plant. It has been known for many years that auxin plays a central role in the functioning of this meristem. Auxin is not homogeneously distributed at the SAM and it is thought that this distribution is interpreted in terms of differential gene expression and patterned growth. In this context, auxin transporters of the PIN and AUX families, creating auxin maxima and minima, are crucial regulators. However, auxin transport is not the only factor involved. Auxin biosynthesis genes also show specific, patterned activities, and local auxin synthesis appears to be essential for meristem function as well. In addition, auxin perception and signal transduction defining the competence of cells to react to auxin, add further complexity to the issue. To unravel this intricate signaling network at the SAM, systems biology approaches, involving not only molecular genetics but also live imaging and computational modeling, have become increasingly important.

Plants continuously generate new tissues and organs through the activity of populations of undifferentiated stem cells, called meristems. Because meristems can modulate their activity, they provide the developmental flexibility that allows plants to adapt their development in reaction to the environment (reviews: Lyndon 1998; Traas and Doonan 2001; Aida and Tasaka 2006; Sablowski 2007).

Distinct meristems exist. Apical meristems, positioned at the tip of the shoots and roots, initiate aerial and underground organs, respectively. Along the stems and roots, more diffuse secondary meristems are responsible for secondary thickening of these structures.

The plant hormone auxin plays an instrumental role in meristem biology and we discuss here its role in a particular meristem, the shoot apical meristem (SAM) that generates all the aerial organs including the floral meristems (Fig. 1A–C). In this context, we limit ourselves to the meristems in angiosperm that have been studied in most detail.

SHOOT APICAL MERISTEM STRUCTURE AND GENETIC REGULATION

All SAMs have a number of basic characteristics in common (e.g., Lyndon 1998; Traas and Doonan 2001). One of the most prominent

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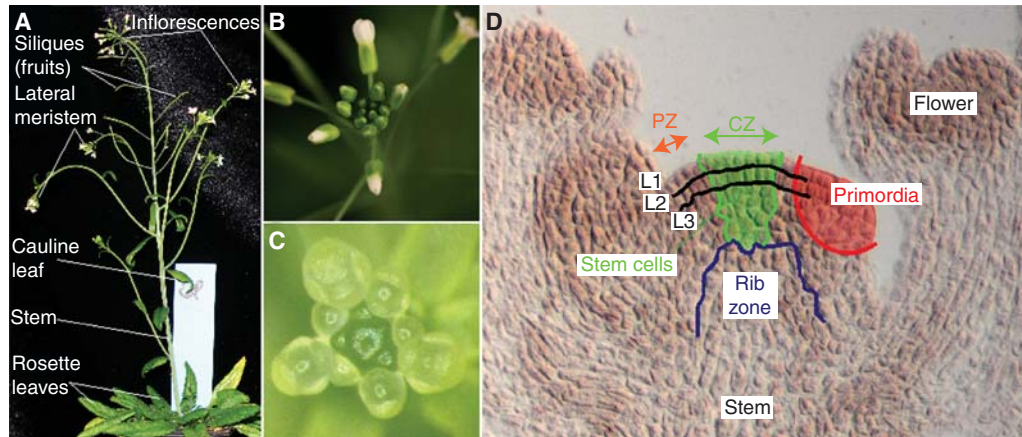


Figure 1. The shoot apical meristem of *Arabidopsis thaliana*. (A) Aerial part of a wild-type plant of the Columbia ecotype (Col-0). The SAM is responsible for the production of rosette leaves and, after floral transition, for the production of the stem, cauline leaves, lateral meristems, and flowers of the inflorescence. (B) Details of the tip of the inflorescence, showing the highly organized positioning of flowers around the main axis (a spiral). (C) A dissected inflorescence meristem. Older flowers have been removed to expose the meristem surrounded by young floral buds. (D) Longitudinal section of an inflorescence meristem showing the layered organization (L1, L2, and L3 cell layers). L1 and L2 are also called the tunica and L3 to the corpus. The functional zones are also represented. At the meristem summit the central zone (CZ) contains the stem cells, whereas primordia are initiated in the peripheral zone (PZ). The rib zone (RZ) produces the internal part of the stem.

features is the presence of a surface layer, called the *tunica* (Fig. 1D). The *tunica*—itself often composed of several sublayers—covers the internal cells of the meristem, collectively called *corpus*. The tunica layers are kept separate as their cells most frequently divide in anticlinal orientations, causing daughter cells to remain in the same layer as their parent. If present, the internal tunica layer—called L2—usually disappears during organ initiation, when L2 cells start to divide in random directions.

Superimposed on this layered organization is a partitioning into zones (Fig. 1D). These zones, characterized by sometimes subtle cytological differences, have well defined functions. At the meristem summit a small group of slowly dividing cells, called the central zone (CZ), ensures a true stem cell function and is responsible for meristem maintenance. The growth rates at the meristem summit usually differ considerably from those at the periphery where cells accelerate their proliferation rates. It is in this peripheral zone (PZ) that new lateral organs are initiated: a restricted number of founder cells increase their proliferation and growth rates

resulting in the formation of a rapidly outgrowing primordium, delimited by the organ boundary region, where cell expansion is reduced.

Extensive genetic screens in different species have identified a network of regulators involved in meristem function, organ initiation, and outgrowth, which is described briefly in the following paragraphs (for reviews: Traas and Doonan 2001; Aida and Tasaka 2006; Sablowski 2007; Rast and Simon 2008). Very similar mechanisms are found in different species, and here we discuss what is known on the best-studied model, *Arabidopsis thaliana*.

A central role in shoot meristem maintenance is played by the transcription factor WUSCHEL (WUS) as in corresponding mutants a SAM is initiated but arrests after having produced a few organs (Laux et al. 1996). Its precise targets and regulators are largely unknown, but it has been well established that it interferes with hormone signaling cascades, in particular cytokinins (Leibfried et al. 2005; Gordon et al. 2009). WUS is involved in a negative feedback loop with the *CLAVATA* receptor kinase signaling cascade (*CLV*), whose activity

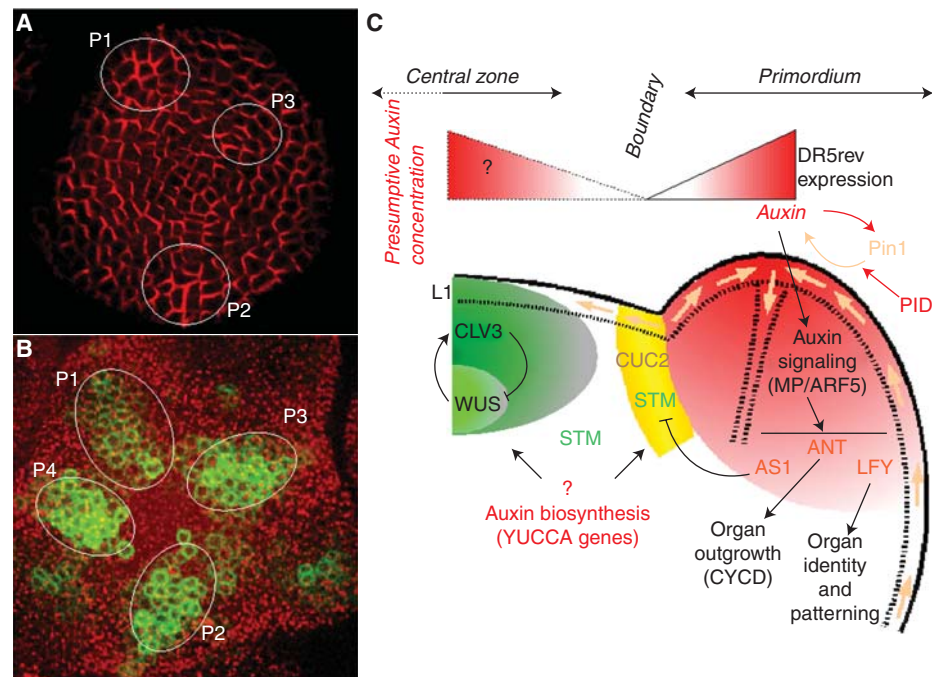


Figure 2. From dynamic transport to patterning: Auxin and organogenesis at the shoot apical meristem of *Arabidopsis thaliana*. (A) Immunodetection of PIN1 efflux carrier in the L1 (top view). The image was obtained by confocal microscopy. Note the subcellular polarized localization of the auxin transporter in most cells. The localization suggests that auxin accumulates in these young organ primordia (named P1, P2, and P3 from the oldest to the youngest organ). Adapted from de Reuille et al. (2006). (B) Expression of the synthetic DR5rev::GFP reporter in the inflorescence meristem (top view). Projection of serial optical sections obtained by confocal microscopy. The green corresponds to the GFP and the red to the autofluorescence of meristematic cells. DR5 expression in young emerging primordia indicates activation of auxin induced-genes. (C) Schematic representation of the role of auxin during organogenesis in the inflorescence meristem of *Arabidopsis thaliana*. See text for details.

limits the size of the pool of stem cells (see Fig. 2C for expression patterns of the genes).

At the periphery of the meristem, the initial recruitment of the organ founder cells appears to be the result of two antagonistic processes. First, the homeodomain protein SHOOTMERISTEMLESS (STM), in combination with several members of the so-called CUP SHAPED COTYLEDON (CUC) family of transcription factors, defines meristematic identity, preventing cells from being recruited by the young organs (e.g., Aida et al. 1999). Therefore, as a new organ is initiated, these meristematic identity genes are switched off in *Arabidopsis*. This involves the transcription factor ASYMMETRIC LEAVES1 (AS1), which maintains the repression of meristem identity factors such as

STM in the developing primordia (Byrne et al. 2000). The meristem either generates leaves and lateral meristems or flowers. The identity of the lateral organs produced depends on the activity of LEAFY (LFY), which, by interacting with different transcription factors such as WUS or AGAMOUS (AG), plays a major role in plant and flower architecture (Lenhard et al. 2001; Lohmann et al. 2001).

The patterning of the flower meristem itself largely depends on transcriptional regulation (for reviews: Krizek and Fletcher 2005; Bowman and Floyd 2008). This includes genes that control organ identity, organ number, organ boundaries, and symmetry. In *Arabidopsis*, for example, *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*), *PISTILLATA* (*PI*) *SEPALLATA1* (*SEP1*),



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and *APETALA3* (*AP3*) direct the development of sepals and petals. Other relevant genes are upstream regulators of these identity genes (e.g., *UNUSUAL FLORAL ORGANS* and *LFY*) but also genes that control the number of sepals and petals formed in each flower (*PERIANTHIA*, *WIGGUM*), genes required to establish organ boundaries in all shoot meristems (*CUC*, genes), and genes that participate in organ patterning (KANADIs, YABBYs, and the *PHABULOSA/PHAVOLUTA/REVOLUTA* group).

Once the founder cell populations of the organs have been identified, they grow out. Our knowledge on the genetic regulation of growth control is more limited and the various signals that integrate and coordinate cell proliferation and expansion remain largely unknown. Some of the connections between the cellular processes and transcriptional regulation have been identified. *AINTEGUMENTA* (*ANT*), for instance, is strongly expressed in the outgrowing organs and acts at least partially via regulating genes involved in cell proliferation like *CYCLIN D* (Mizukami and Fischer 2000). In petals, several genes control growth by affecting cell proliferation and/or cell expansion (Anastasiou and Lenhard 2007). Some of these genes (i.e., *JAGGED*, *ANT*, and *ARGOS*) affect petal growth by positively regulating cell proliferation (Mizukami and Fischer 2000; Hu et al. 2003; Dinneny et al. 2004), whereas other genes (*BIGBROTHER*, *KLUH*, and *DA1*) control final organ size by negatively regulating the duration/period of cell proliferation (Disch et al. 2006; Anastasiou et al. 2007; Li et al. 2008). *BIGPETAL*, which is regulated downstream of the flower organ identity genes, limits petal growth by controlling cell expansion (Szecsi et al. 2006).

In addition to transcriptional control, post-transcriptional regulation by microRNAs (miRNAs) plays an important role in meristem function. These include, for example, the miR164 and miR156/157 families, which respectively target the *CUC* and *SPL* genes (Peaucelle et al. 2007; Wang et al. 2009).

How is this complex regulatory network coordinated? It has been known for many years that auxin plays a central role in meristem function and organ formation. Auxin is not

homogeneously distributed at the SAM and it is thought that this distribution is interpreted in terms of differential gene expression and patterned growth. Because the distribution of auxin is largely under the control of auxin transporters, we first discuss auxin transport at the shoot apex.

AUXIN TRANSPORT AND SAM FUNCTION

Auxin Influx and Efflux Carriers Control Auxin Distribution at the SAM

Okada et al. (1991) identified an *Arabidopsis* mutant called *pin-formed 1* (*pin1*) in reference to its needle-like inflorescence stem, unable to initiate flowers. Identification of the gene showed that it encoded a transmembrane protein (Galweiler et al. 1998) and there is now overwhelming evidence that the PIN1 protein is the founding member of a family of auxin carriers that transport auxin across membranes (for review: Petrasek and Friml 2009). More particularly, PIN1 and closely related family members are involved in auxin efflux out of the cells. PIN1 is strongly expressed in the SAM, where it is mainly found in the tunica and vascular tissues (Fig. 2A). The activity of other PIN-members at the SAM has not been analyzed in detail. Expression studies show that at least PIN 2 and 7 are active in the shoot apex but single mutations in these genes do not significantly perturb meristem function (Muller et al. 1998; Friml et al. 2003). The *pin1* phenotype can locally be complemented by local, external applications of high concentrations of auxin, which are able to induce the formation of flower buds (Reinhardt et al. 2000). These applications indicate that high auxin concentrations are required for the initiation of a new organ and, importantly, that PIN transporters are required for the creation of such auxin maxima. Direct evidence demonstrating the existence of auxin maxima at the periphery of the meristem are still lacking because of the absence of a true auxin sensor. However, this scenario is further corroborated by the activation of the synthetic DR5 auxin reporter in the young organs (Fig. 2B). DR5 is composed of a fusion of a synthetic



promoter consisting of several auxin response elements (AuxRE) and a minimal 35S promoter with a reporter gene (Sabatini et al. 1999). The AuxREs corresponds to the DNA sequences bound by the AUXIN RESPONSE FACTORS (ARFs; see later for more details) and expression of the reporter gene thus suggests activation of auxin-induced genes during the earlier stages of organ initiation (Benkova et al. 2003; de Reuille et al. 2006; Smith et al. 2006). In addition, the polarity of the PIN transporters has been analyzed to identify the putative direction of auxin fluxes. This has confirmed the hypothesis that auxin fluxes are directed toward the young primordia (Benkova et al. 2003; Reinhardt et al. 2003).

Second sets of transporters associated with auxin distribution at the SAM are the AUX/LAX influx carriers. AUX1, the founding member of the gene family (Bennett et al. 1996), is expressed in the L1 surface layer of the shoot apical meristem (Reinhardt et al. 2003). The protein seems to be evenly localized over all membranes, indicating that the protein is not involved in the creation of hormone fluxes. Instead, it might rather concentrate auxin at the meristem surface. Somewhat surprisingly, its loss of function has only a relatively mild phenotype in the shoot, including perturbations in phyllotactic patterns. The *AUX1* sequence shows a high degree of similarity with three other sequences in the *Arabidopsis* genome, named *LAX1*, *LAX2*, and *LAX3* (Like *AUX1*) (Swarup and Bennett 2003), but even the quadruple mutant is still able to produce a viable, fertile plant albeit with important changes in its architecture (Bainbridge et al. 2008). It is therefore conceivable that other proteins, such as certain P-glycoprotein transporters (PGPs), which have also been associated with auxin import, share a redundant function with the members of the LAX family in the meristem (For review: Boutte et al. 2007). Another clue on the precise role of AUX1 comes from a double *pin1/aux1* mutant. Auxin applications do not induce single flowers on the apex of such a double mutant, but rather very large, fused organs, suggesting that somehow AUX1 is required for the restriction of organ boundaries (Reinhardt

et al. 2003). Altogether, the available data indicate that the formation of local auxin maxima mainly depends on the action of PIN exporters at the meristem surface. AUX and LAX proteins would facilitate organ positioning, probably by guaranteeing a sufficient supply in the L1 layer.

Using Modeling to Explore Polar Auxin Transport Regulation: Canalization or Up-the-Gradient?

The distribution of PIN at the meristem surface is complex and it is not obvious from simple visual inspection what the predicted auxin fluxes would be. Therefore, a set of careful localization studies were performed and the properties of the cellular transport networks analyzed using computer simulations (de Reuille et al. 2006). This confirmed that PIN directs auxin to the sites where young primordia are being formed. The same computer simulations also identified additional properties of the transport network, and in particular a still undefined role for the meristem summit in auxin redistribution was proposed (de Reuille et al. 2006).

What coordinates auxin fluxes in the meristem? The complex and dynamic patterns of PIN distribution could suggest very intricate regulatory mechanisms. Computer models, however, have shown that the actual basis of the observed transport patterns could be very simple. Jönsson et al. (2006) and Smith et al. (2006) proposed models where cells check the auxin concentrations in their direct environment and subsequently pump auxin toward neighbors with a higher concentration, i.e., against the gradient. Because these patterning processes require the interaction of hundreds of cells, it is impossible to estimate on a purely intuitive basis if a particular scenario is plausible or not. Therefore, computational modeling was used as a powerful means to test this hypothesis. Interestingly, these models showed that transport “against the gradient” is sufficient to reproduce realistic PIN1 patterns and to generate different types of phyllotactic patterns (Jonsson et al. 2006; Smith et al. 2006).

It should be noted that, although these models show that the mechanism is plausible,

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they by no means provide absolute proof. Indeed, other models are able to explain the distribution of auxin transporters. Another hypothesis, for instance, was based on the pioneering work of Sachs who proposed the existence of a positive feedback between flux and transport (Sachs 1969). It was subsequently shown that this mechanism is able to amplify small fluxes and can potentially create canals of auxin between hormone sources and sinks. A range of experiments supports the canalization hypothesis, at least in the inner tissues of the plant where it can account for the formation of venation patterns (Scarpella et al. 2004; Sauer et al. 2006; Scarpella et al. 2006). The existence of canalization would imply the coexistence of two radically different mechanisms for PIN allocation to the membrane, one based on flux sensing (in the inner tissues) and the other on local concentration sensing (at the meristem surface). Therefore, Stoma and colleagues (2008) tested whether canalization could potentially also account for the behavior of auxin transporters at the shoot apical meristem surface. Using a dedicated computer simulation tool, they showed that this was indeed the case, thus providing a unifying concept for the control of auxin distribution in the plant (Stoma et al. 2008). More recently, Bayer and coworkers tested the alternative scenario where both modes of auxin transport (i.e., up-the-gradient and canalization) coexist. One or the other mechanism would prevail, depending on the absolute auxin concentrations (Bayer et al. 2009). This model also reproduced realistic distributions of PIN proteins. Further experiments are now required to distinguish between flux-based polarization and other hypotheses. Although these models remain to be tested, notably through the identification of the molecular mechanism controlling PIN1 polarity, they show that very simple local behaviors of individual cells can generate the complex overall patterns we observe.

Orienting Auxin Transport at the SAM: The Role of PID

Concepts like canalization are still relatively abstract and could represent a combination of

processes. Indeed, although the exact mechanisms that control the dynamics of PIN1 polar localization are still unknown, accumulating evidence indicates that it depends on multiple processes, such as membrane traffic and cytoskeleton organization (for review: Kleine-Vehn and Friml 2008). A striking set of data also points at the importance of phosphorylation and the PINOID (PID) serine-threonine protein kinase (Christensen et al. 2000; Benjamins et al. 2001). *Arabidopsis* mutants perturbed in the *PID* gene have *pin*-like inflorescences. Interestingly, this goes along with an apical-to-basal shift in PIN1 polarity (Friml et al. 2004). Accordingly, in the root, ectopic expression of PID induces a basal-to-apical shift in PIN2 and PIN4 polarities. The results so far indicate that the PID kinase controls PIN localization via direct phosphorylation of the transporter (Michniewicz et al. 2007). Interestingly, the kinase does not seem to affect the polarized localization per se, but rather decides on what particular membrane the carrier will be localized. Lee and Cho (2006) also showed that PID may facilitate trafficking of PIN to the plasma membrane, suggesting that it may not only affect the polarity of PIN1 but also the dynamics of polarity changes in meristematic cells (Lee and Cho 2006). Antagonist regulation of PIN1 by PP2A phosphatase has been shown (Michniewicz et al. 2007) and PID activity itself depends on its autophosphorylation status (Zegzouti et al. 2006). Precise modulation of both PIN1 and PID phosphorylation status is thus an essential regulation level of PIN1 localization in the meristem. It was recently shown that PINs are initially delivered to the plasma membrane in a nonpolar manner and that their polarity is established by subsequent endocytic recycling (Dhonukshe et al. 2008). It is thus possible that PID could be involved in directing PIN1 to specific membranes, thus participating in controlling the dynamics of the PIN1 network in the meristem.

Other genes are involved in the PID pathway, like *NPY1/ENP/MAB4*, a NPH3-like protein first identified as an enhancer of *PID* during cotyledon development (Trembl et al. 2005; Furutani et al. 2007). Mutations in *NPY1*

induce pin-like inflorescence in the *yuc1 yuc4* (*yucca*) double mutant affected in auxin biosynthesis ([Cheng et al. 2007b]; see part 4 for discussion on the role of the YUCCA genes). NPY1 is expressed in the L1 at the meristem and accumulates in the young organs (Cheng et al. 2007b; Furutani et al. 2007). The inactivation of NPY1 together with two of its closest homologs also completely abolishes organ initiation at the SAM (Cheng et al. 2008). Although the exact role of NPY1 and its homologs still needs to be established, these results suggest that the NPY protein mediates or facilitates PID action on PIN1 localization.

It is interesting to note that *PID* is expressed at a higher level in the frontiers that separate organs from the meristem (Furutani et al. 2004). It is precisely at these boundaries that PIN undergoes important relocalizations (Heisler et al. 2005). *PID* could therefore play a decisive role in organ separation.

THE ROLE OF AUXIN METABOLISM AT THE SAM

Although the role of polar auxin transport during developmental processes, notably in the shoot meristem, has been extensively studied, little attention has been paid to the role of auxin metabolism until recent years. However, local concentrations of auxin in the shoot meristem are expected to be under the control of the combined action of polar auxin transport and auxin biosynthesis, but also of auxin catabolism and conjugation. Auxin metabolism is still largely under investigation and its complexity provides one historical reason for focusing primarily on polar auxin transport. Nevertheless, recent studies have identified mutants in key biosynthetic enzymes that highlight the importance of local auxin biosynthesis in the shoot meristem and underline the need to consider this parameter when studying the role of auxin in the shoot meristem and more generally in plant development.

Several excellent reviews have described our current knowledge on auxin metabolism (e.g., Ljung et al. 2002; Woodward and Bartel 2005).

We will therefore focus mainly on its importance in shoot apical meristem function.

Local Auxin Biosynthesis is Essential for Shoot Apical Meristem Function

IAA biosynthesis occurs mostly through a tryptophan (Trp)-dependent pathway and involves four parallel and partly interdependent pathways named after the main intermediates: the indole-3-pyruvic acid (IPA), tryptamine (TAM), indole-3-acetaldoxime (IAOx), and indole-3-acetamide (IAM) pathways (Woodward and Bartel 2005). The recent identification of the YUCCA (YUC) family of auxin biosynthetic genes encoding flavin monooxygenases regulating the TAM pathway has been instrumental in demonstrating that auxin biosynthesis is not homogenous in a given tissue and that a fine control of auxin biosynthesis in the shoot apex plays a key role in the regulation of the activity of the shoot and floral meristems.

The YUC family has 11 members and YUC1, its founding member, was isolated based on an increase in hypocotyl length in an activation-tagging screen (Zhao et al. 2001). The *yuc1-D* gain-of-function mutant displayed typical auxin overproduction phenotype, such as epinastic cotyledons and long hypocotyl, short roots and increased apical dominance. Based on in vitro biochemical assays and on resistance of the mutant to the toxic tryptophan analog, 5-methyl-tryptophan, it was proposed that YUC1 catalyzed the oxidization of tryptamine to *N*-hydroxyl-tryptamine, a rate-limiting step in the tryptophan-dependent auxin biosynthesis tryptamine pathway (Zhao et al. 2001). The importance of YUC-dependent local auxin biosynthesis in organ initiation was revealed by the analysis of multiple loss-of-function *yucca* mutants in *Arabidopsis* (Cheng et al. 2006; Cheng et al. 2007a). Although none of the single mutants showed obvious phenotypes, several double and triple mutant combinations and the quadruple mutant of *yuc1*, *yuc2*, *yuc4*, and *yuc6* displayed strong inflorescence and floral developmental defects as well as alterations of leaf vasculature (Cheng et al. 2006). These floral and vascular phenotypes are reminiscent of

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those observed in the *pin1*, *pid*, and *ettin/arf3* mutants (Okada et al. 1991; Bennett et al. 1995; Sessions et al. 1997). In the quadruple *yuc* mutant, pin-like structures were also observed, indicating that these four *YUC* genes are involved in floral development from the initiation of the flower onwards. The analysis of the expression pattern of the *YUC* genes further suggested that they regulate flower initiation and development through the spatial and temporal control of auxin biosynthesis. Direct evidence that the developmental defects in the multiple *yuc* mutant were caused by a local decrease in auxin biosynthesis was shown by complementing the *yuc1yuc4* double mutant with a bacterial auxin biosynthesis gene under the control of the *YUC1* promoter (Cheng et al. 2006). In addition, Cheng et al. (2007) showed through the analysis of combinations of mutants in *yuc1*, *yuc4*, *yuc10*, and *yuc11* that auxin synthesized by the *YUC* proteins is also necessary for leaf development.

The temporal and spatial regulation of *YUC* genes thus appears to provide a mechanism for fine-tuning the concentration of auxin during organ initiation and development at the shoot apex. Mutants in *YUC* homologs of petunia, maize, and rice (Tobena-Santamaria et al. 2002; Fujino et al. 2008; Gallavotti et al. 2008) exhibited similar developmental defects, indicating that the regulation of the TAM pathway for auxin biosynthesis is a conserved mechanism for modulating auxin in the angiosperms. The identification of TRYPTOPHAN AMINOTRANSFERASE of ARABIDOPSIS 1 (*TAA1*), a long-predicted key enzyme in the indole-3-pyruvic acid (IPA) pathway, and its paralogs (TRYPTOPHAN AMINOTRANSFERASE RELATED or *TAR*) has recently shown that spatial regulation of auxin biosynthesis through this second Trp-dependent pathway might also play a similar role during development (Stepanova et al. 2008; Tao et al. 2008). Stepanova et al. (2008) notably found that the double *taa1/tar2* mutant displays a severe reduction of growth of the aerial tissues and alterations of flower development, suggesting a role in floral organ initiation. As for the *YUC* genes, *TAA1* expression is restricted to specific parts of the flower and the gene might

be involved in the local regulation auxin biosynthesis.

At least two nonredundant auxin biosynthesis pathways are thus implicated in organ initiation at the shoot or floral meristem. The phenotype obtained on inactivation of these pathways indicates that they are necessary to control locally auxin homeostasis at the shoot meristem and that auxin transport is probably not the only limiting mechanism for generating the spatial variations in auxin concentration implicated in lateral organ initiation.

Integrating Auxin Metabolism and Transport at the Shoot Meristem: A Challenge for the Future

The results discussed in the previous paragraphs suggest that the integrated action of both auxin transport and metabolism define where and when auxin will accumulate. Ljung et al. (2001) showed the existence of a feedback mechanism between biosynthesis and transport by measuring auxin concentration and synthesis rates on treatment of young *Arabidopsis* roots with NPA (Ljung et al. 2001). Cheng et al. (2007) suggest that such a feedback mechanism is also active in the shoot meristem because combinations of the *pin1* mutation with *yuc1* and *yuc4* enhances *pin1* defects, blocking almost completely leaf and flower initiation. A similar phenotype is obtained by combining *aux1* with *yuc1*, *yuc2*, *yuc4*, and *yuc6* (Cheng et al. 2007a). Cross-regulations between auxin transport and biosynthesis could be a key feature explaining the robustness of auxin gradients (Weijers et al. 2005) and it would be particularly instructive to analyze the effect of incorporating auxin production and inactivation on the stability of phyllotaxis models. Further work on the spatial control of auxin biosynthesis will probably be essential in this context.

FROM AUXIN SIGNAL TRANSDUCTION TO MORPHOGENESIS AT THE SHOOT APEX

The action of auxin does not only depend on the regulation of its synthesis or transport, but the competence to react to auxin seems also to

be controlled in time and space. We now discuss the regulation of auxin signal transduction in the shoot meristem before considering how auxin interacts with the gene networks underlying organ outgrowth.

Spatial Modulation of Auxin Signal Transduction is Involved in Meristem Function

Both biochemical and genetic approaches have identified the members of the Aux/IAA and ARF family of transcriptional regulators as major effectors of auxin signal transduction (for review: Leyser 2006). There are 29 Aux/IAA genes that encode mostly short-lived repressors of auxin-inducible genes. The 23 ARF proteins can be either activators (Q-rich ARFs) or repressors of transcription. Aux/IAA and ARF proteins are able to form homo- and heterodimers both within and between the families. The instability of the Aux/IAAs is intrinsic to the so-called domain II, which interacts directly with SCF-like ubiquitin protein ligases harboring the TIR1 F-box protein or one of the three Auxin-related F-box (AFB) proteins (Dharmasiri et al. 2005a; Dharmasiri et al. 2005b; Kepinski and Leyser 2005; Tan et al. 2007). TIR1 and the AFBs act directly as auxin receptors and their activation leads to auxin-dependent degradation of Aux/IAAs. The current simplified model for auxin transduction is that Aux/IAAs dimerize with the Q-rich ARFs. These complexes bind to auxin-inducible genes, thus preventing transcription. By promoting the degradation of the Aux/IAAs by the SCF, auxin would allow the ARFs to activate transcription of target genes by binding to the AuxREs present in their promoter.

When *pin1* meristems are treated with exogenous auxin, all the cells at the periphery of the meristem are competent to respond to auxin to initiate organs (Reinhardt et al. 2000). However, auxin is not able to induce organs at the central zone of the meristem. Mutation in *MONOPTEROS/ARF5* induces a phenotype similar to *pin1* and blocks the ability of the meristematic cells to respond to exogenous auxin (Hardtke and Berleth 1998; Reinhardt et al. 2003).

Because *MP/ARF5* is only expressed at the meristem periphery (Hardtke and Berleth 1998), the competence for organ initiation at the periphery of the meristem thus depends, at least in part, on a spatial modulation of auxin signal transduction. Despite an extensive genetic analysis of the ARF and Aux/IAA families (Okushima et al. 2005; Overvoorde et al. 2005), no other ARF and no Aux/IAA has been implicated in shoot meristem function, although indirect evidence suggests that several ARFs could be involved (Hardtke 2004; Pekker 2005; Mallory 2005). The lack of genetic evidence could be because of the demonstrated redundancy between these transcription regulators (Ellis 2005; Pekker 2005; Okushima 2005). It will therefore be important to generate multiple mutant combinations for ARFs as well as dominant negative mutant in all *Aux/IAA* members. Together with an exploration of the expression patterns of these two gene families, this should give valuable information of the role of signal transduction modulation in the dynamics of auxin responses in the meristem.

Induction of the Morphogenetic Program by Auxin in the Shoot Meristem

The analysis of cell identity in the meristem of the *pin1* mutant gave the first clues concerning the role of auxin in the control of cell identity at the meristem (Vernoux et al. 2000). Although meristem structure and maintenance were not severely affected in the mutant, major alterations occurred at the periphery of the *pin1* meristem, where organ initiation should occur. A ring-like domain expressing two early markers of organ initiation, *LFY* and *ANT*, but also the boundary marker *CUC2* was observed around the central zone. This showed that the zone at the meristem periphery has a hybrid identity in *pin1*. These results also implied that auxin levels control the identities of the cells at the periphery of the meristem, thus impacting organ separation, positioning, and outgrowth. Analysis of the genetic interactions between *PIN1* and *MP/ARF5* further suggested a differential regulation of the *CUC* genes by *PIN1* and *MP* during embryogenesis (Aida et al. 2002). Taken

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together, these data suggest a model where auxin, through *PIN1* and *MP*, could regulate patterning at the meristem partially through the control of *CUC* gene expression (Fig. 2C).

More recently, live-imaging of *Arabidopsis* inflorescence meristems allowed detailed analysis of the sequence of gene activation in relation to auxin transport and responses (Heisler et al. 2005). These authors used *PIN1*-GFP translational fusion in conjunction with the auxin-responsive *DR5::VENUS* marker, to show that the convergence of *PIN1* proteins toward the site of the new organ is likely the first event of organogenesis and is immediately followed by auxin-induced transcription. By combining multiple GFP-protein fusions, their work further suggests that the *KNOX* gene *STM* and *CUC2* are down-regulated in the early primordia, whereas they are up-regulated in the frontiers in response to specific auxin concentration and/or responses. This elegant analysis not only linked auxin patterns at the meristem to developmental patterning but also supported the proposed role of *PIN1* in *CUC* regulation discussed earlier. In addition to the effects on *STM*, Hay et al. (2006) demonstrate that *BREVIPEDICELLUS* (*BP*), another *KNOX* gene, is ectopically expressed in the leaves of a *pin1* mutant, whereas the *bp* mutation slightly suppresses both *pin1* and *pid* mutants (Hay et al. 2006). These data support a scenario in which the expression of *KNOX* genes is first repressed in the incipient primordium by an auxin-dependent mechanism, and is later maintained in a repressed state *via* the action of regulators such as *AS1* (Fig. 2C).

Auxin is not only involved in organ initiation but is also associated with the establishment of symmetry. *In vivo* imaging suggests that auxin maxima precede and might control the establishment of adaxial/abaxial symmetry by *FILAMENTOUS FLOWER* and *REVOLUTA* (Heisler et al. 2005). Such a role was further supported by genetic studies, identifying *ETTIN*/*ARF3* and *ARF4* as regulators of organ asymmetry (Pekker et al. 2005). Another link between auxin and organ symmetry involves members of the *KANADI* (*KAN*) gene family, which regulate abaxial identity and laminar growth of

lateral organs (Bowman and Floyd 2008). Mutations in *ARF3/ETTIN* were isolated as second site suppressors of ectopic *KAN* expression (Pekker et al. 2005). Further genetic and expression analysis indicated that *ARF3/ETTIN* and *ARF4* mediate the *KAN* pathway but that *KAN* does not regulate *ARF3* and *ARF4* activation in lateral organs. *ARF3* and *ARF4* are expressed during the early stages of organ initiation (Sessions et al. 1997; Pekker et al. 2005), indicating that modulation of auxin activity is likely cooperatively involved with *KAN* in setting up adaxial/abaxial organ polarity (Pekker et al. 2005).

INTEGRATION OF HORMONE SIGNALING AT THE SHOOT APEX

As in other tissues, *SAM* function relies on the combinatorial action of several hormones (Shani et al. 2006). We review here briefly the best-characterized hormone interactions involving auxin.

Studies on plant tissue regeneration have shown that a high cytokinin (*CK*) to auxin ratio can trigger the initiation of shoot meristems from undifferentiated callus but the molecular basis for such interactions in the shoot are just starting to emerge (Gordon et al. 2007; Gordon et al. 2009). In the last few years, it has been shown that high *CK* signaling is essential for maintenance of the meristem through a direct effect on stem cell activity. However, the identification and characterization of the *aberrant phyllotaxy1* (*abphyl1*) mutant of maize suggest that *CKs* contributes to organ initiation together with auxin. This mutant shows decussate phyllotaxis, whereas distichous arrangement are usually observed in wild type plants (Jackson and Hake 1999). *ABPHYL1* encodes a cytokinin-inducible *RESPONSE REGULATOR*, a primary cytokinin response gene likely to act in a negative feedback loop to lower *CK* signaling in the leaf primordia (Giulini et al. 2004). In *abphyl1* mutants, auxin contents are lower than in wild type and *zmPIN1* expression in the incipient leaf primordia is also greatly reduced (Lee et al. 2009). Unexpectedly, *zmPIN1* is rapidly induced in the meristem on exogenous cytokinin



applications, suggesting a complex regulation of PIN1 expression by CK. These observations in maize are partly consistent with the effect of CK on PIN expression observed in the *Arabidopsis* root meristem (Laplaze et al. 2007; Dello Ioio et al. 2008). Together with other recent findings, such as the regulation of CK degradation by auxin in leaf primordia during shade avoidance (Carabelli et al. 2007) or the activation of the CK receptor AHK4 by auxin in hypocotyl explants during the first steps of shoot regeneration (Gordon et al. 2009), these data also further support a possible fundamental role for auxin-CK interactions during organ formation and meristem function.

Auxin is also associated with another key class of hormones in the shoot meristem, the gibberellins (GA). The concentration of active GA is mainly controlled by GA 20-oxydases and GA 3-oxydases, required for GA biosynthesis, and by GA 2-Oxydases, which control GA deactivation. These enzymes are encoded by gene families, some of which show very specific expression patterns in the shoot meristems. In particular, biosynthetic enzymes are excluded from the meristem center and restricted to young organs, whereas deactivation enzymes are expressed at the base of the SAM, below the rib zone (Jasinski et al. 2005; Hu et al. 2008; Rieu et al. 2008). Auxin treatments on seedlings suggest that eight out of 13 GA oxydases (both activating or deactivating) expressed in seedlings are transcriptionally regulated by auxin (Frigerio et al. 2006). Among those, at least GA20ox1, GA2ox2, and GA2ox4 are expressed in the vegetative SAM and could be targets of auxin signaling in the shoot, although this remains to be shown.

GENERAL CONCLUSION

In the last 10 years, auxin has emerged as a fundamental mobile signal in the shoot meristem as it triggers organ initiation at the periphery of the meristem. The data available so far clearly show that the dynamics of the PIN1 transport network generates localized auxin maxima that are responsible for organ initiation and changes in cell identity. In addition, there is

accumulating evidence for an important role of local auxin biosynthesis, although very little is known on how this influences auxin distribution. Likewise, the role of other important regulators of auxin signaling, such as the putative receptor ABP1 (Braun et al. 2008), remains to be determined. The main challenge for the future will be to understand how auxin transport is integrated with auxin biosynthesis and other hormone signaling in order to coordinate cell behavior and differentiation in this complex dynamic structure. The use of systems biology approaches, together with the development of new biological tools to monitor auxin concentration and signaling in live tissues, will likely be the key to reach a new level in our understanding of hormonal signal integration at the shoot meristem.

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