Minireview

Cytokinin and auxin intersection in root meristems

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Abstract

The hormone cytokinin promotes cell differentiation in plant roots by repressing both auxin transport and responses to auxin at the boundary between the meristem and the root elongation zone.

In plants, populations of undifferentiated cells called meristems serve as the source of all new growth throughout post-embryonic development. Meristem size, or the number of undifferentiated cells in the meristem, is maintained by balancing stem-cell replenishment (through cell division) with cell differentiation and elongation to form new tissues and organs. In the *Arabidopsis* root, meristem maintenance is controlled by the small-molecule hormones auxin and cytokinin, which affect cell division and cell elongation. Although auxin and cytokinin have long been known to interact during development, the molecular mechanisms of these interactions have been elusive. In a recent issue of *Science,* Sabrina Sabatini and colleagues (Dello Ioio *et al.* [1]) have elucidated a simple molecular mechanism by which cytokinin regulates meristem size in the *Arabidopsis* root by antagonizing auxin signaling in the transition zone, the region where cells leave the meristem to differentiate and elongate.

Auxin and cytokinin have contrasting roles in root meristems. Auxin is required for meristem cell division: application of exogenous auxin increases root meristem size, for example, whereas cytokinin reduces it [2,3]. Basipetal transport and lateral distribution of auxin are required for stem-cell replenishment, as plants carrying mutations in three members of the *PIN-FORMED* (*PIN*) family of auxin-efflux carrier proteins have reduced meristem size [4]. In previous work, Sabatini and colleagues [3] had shown that endogenous cytokinin is required to control stem-cell division, as plants defective in cytokinin biosynthesis had expanded meristems. This phenotype is also observed in plants with mutations in *ARABIDOPSIS HISTIDINE KINASE3* (*AHK3*), which encodes a cytokinin receptor, or *ARABIDOPSIS RESPONSE REGULATOR1* (*ARR1*) or *ARABIDOPSIS RESPONSE REGULATOR12 (ARR12)*, B-type response regulators that encode transcription factors that specifically activate 'cytokinin-responsive' genes [5] (Figure 1a). Together with other studies depleting cytokinin in the root transition zone [6], this suggested that cytokinin controls meristem size by acting through the *AHK3/ARR1, ARR12* pathway to attenuate auxin-dependent stem-cell division in the root meristem.

ARR1 targets an auxin-response repressor

Dello Ioio *et al.* [1] now elucidate the details of this cytokinin-auxin interaction. They first asked whether ARR1 is sufficient for regulation of meristem growth. By transforming plants with a construct encoding a glucocorticoid-inducible form of ARR1, they induced ARR1 overexpression and found that this is sufficient to reduce meristem size, similar to the effect of exogenous cytokinin. Previously identified targets of ARR1 include the auxin-response repressor gene *SHORT HYPOCOTYL2* (*SHY2*) [7], which is particularly interesting as it is required for normal root growth [8]. The hormone auxin acts by enabling the transcription of specific auxin-response genes; it does this by accelerating the degradation of repressor proteins that suppress auxin-response genes (see [9] and references therein) (Figure 1b). SHY2 is one of these repressor proteins, a member of the AUX/IAA family of transcriptional repressors [10,11].

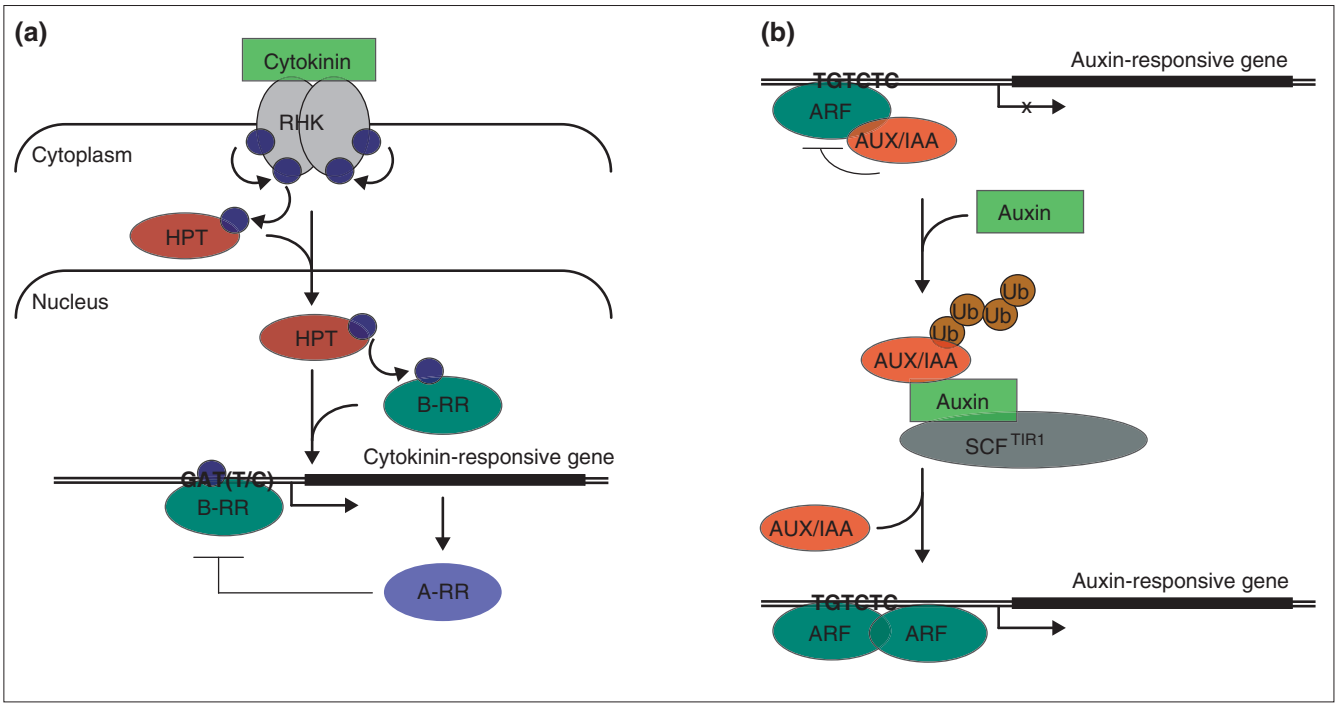

Figure 1
Cytokinin and auxin signaling in *Arabidopsis*. Single lines indicate cell membranes; double lines represent the chromosome; bent arrows indicate positions of transcription initiation. **(a)** Cytokinin binding to a receptor histidine protein kinase (RHK) such as AHK3 triggers kinase autophosphorylation and initiates a phosphorelay cascade [5]. The phosphoryl group (blue sphere) transfers to a receiver domain in the receptor and subsequently to a histidine phosphotransfer protein (HPT), triggering HPT translocation to the nucleus. There, the phosphorylation is relayed to an *Arabidopsis* response regulator (ARR) such as ARR1. B-type RRs (B-RR) activate transcription of cytokinin-responsive genes, some of which contain a GAT(T/C) DNA sequence motif [7]. Cytokinin-responsive A-type RRs (A-RR) act to repress cytokinin signaling. **(b)** Auxin signaling is based on auxin-dependent, proteasome-mediated degradation of AUX/IAA repressors (see [9] and references therein). AUX/IAA proteins dimerize with and repress the activity of transcription factors in the *AUXIN RESPONSE FACTOR* (*ARF*) family, which bind TGTCTC-containing DNA sequence elements in promoters of auxin-responsive genes. Auxin-dependent gene expression is mediated by the release of ARF proteins from AUX/IAA repression as a result of proteasome-mediated degradation of AUX/IAA proteins. Auxin serves as the switch by binding to an F-box protein such as TRANSPORT INHIBITOR RESPONSE1 (TIR1) and enhancing its interaction with AUX/IAA proteins, increasing the rate of AUX/IAA ubiquitination (Ub) by the Skp1-Cul-F-box E3 ubiquitin ligase complex SCF^{TIR1}.

Dello Ioio *et al*. show that *SHY2* is expressed in the vascular tissue of the root transition zone (Figure 2a), and is induced by cytokinin. They also found that loss of SHY2 in the *shy2-31* mutant results in larger root meristems (mimicking the effects of auxin application) and resistance to cytokinin treatment. Conversely, plants carrying the gain-of-function *shy2-2* allele have smaller meristems (mimicking the effects of ARR1 overexpression) and are hypersensitive to treatment with cytokinin. The *shy2-2* mutation acts to stabilize SHY2, resulting in increased protein levels. These results indicate that *SHY2* is both necessary and sufficient for cytokinin-mediated control of meristem size, and that it forms a point of intersection of the cytokinin and auxin signaling pathways. It is important to note, however, that this work does not exclude other members of the AUX/IAA family from involvement in meristem size control.

SHY2 expression and cytokinin-inducibility are lost in *arr1* mutant plants, suggesting that *SHY2* acts downstream of *ARR1*. In support of this, the meristems of *arr1* and *shy2arr1* double-mutant plants are similar in size. Dello Ioio *et al*. [1] use chromatin immunoprecipitation and electrophoretic mobility shift experiments to show that the ARR1 protein interacts with the *SHY2* promoter *in vivo* and with a GATC-containing ARR1-binding consensus sequence in the *SHY2* promoter *in vitro*. Finally, the authors analyzed meristem size in *shy2* loss-of-function mutants overexpressing *ARR1*. In the *shy2* mutant, *ARR1* overexpression does not trigger the meristem size decrease observed in the wild-type background, confirming that SHY2 is necessary for cytokinin-mediated meristem size control via ARR1.

Cytokinin represses PIN expression through SHY2

In their 2007 study [3] Dello Ioio *et al*. noted that cytokinin is required in the vascular transition zone for differentiation of all cell types in the zone, leading them to hypothesize that cytokinin could act in this region to repress auxin signaling. Indeed, they observed that auxin transport by PIN proteins is required for the increase in meristem size resulting from cytokinin depletion in the vasculature [3]. In the current

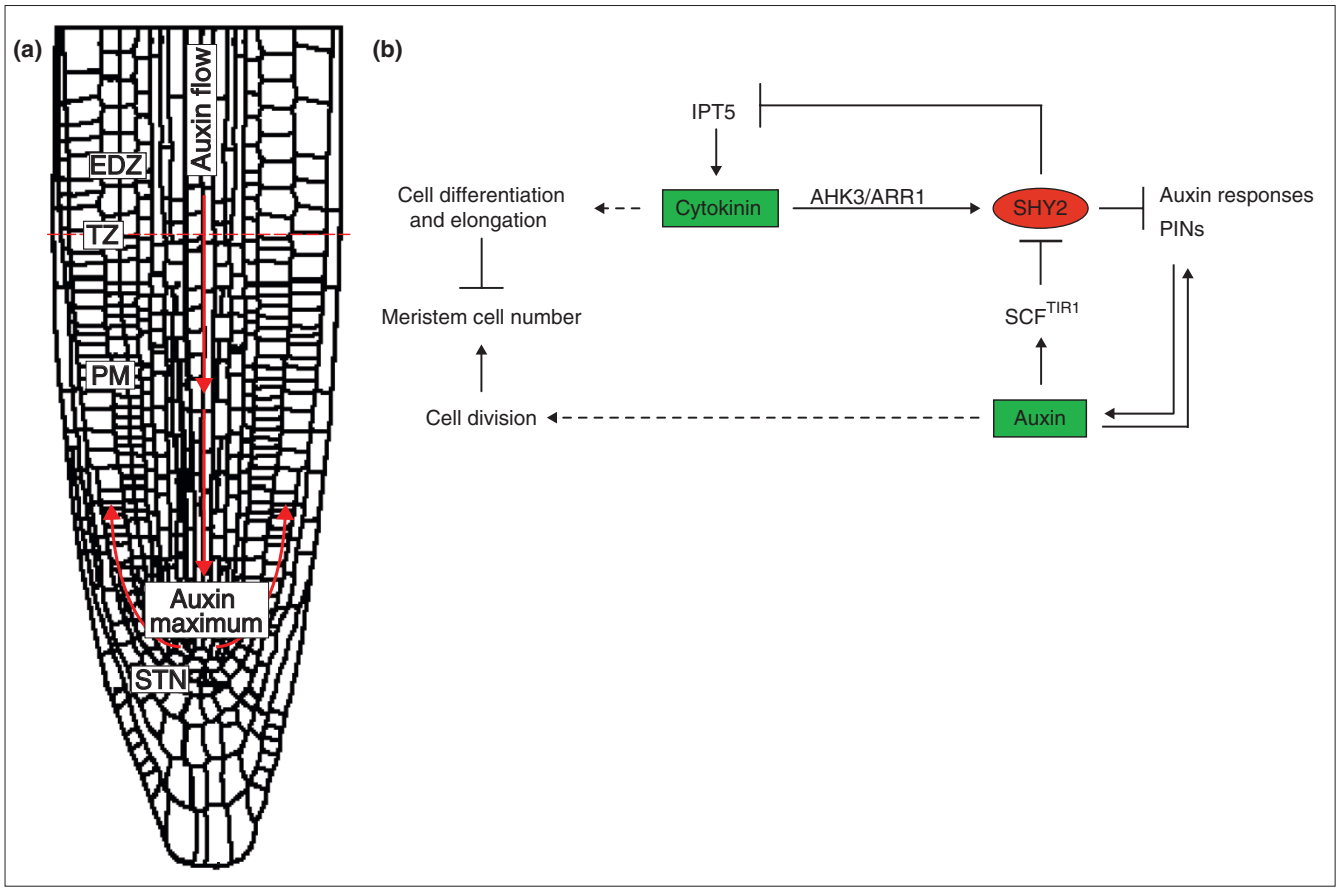

Figure 2
Model for positioning the root transition zone. **(a)** In the *Arabidopsis* root, basipetal auxin transport generates an auxin maximum at the stem-cell niche (STN) where it is required for stem-cell specification [4,16]. Cell division is confined to the proximal meristem (PM), and cells differentiate and elongate in the elongation differentiation zone (EDZ). The transition zone (TZ) is the boundary between dividing and expanding cells. **(b)** Meristem size, defined as the number of cells in a cell file in the proximal meristem, is determined by rates of cell division in the meristem and differentiation at the transition zone. Perturbations favoring cell division increase meristem size, whereas those favoring differentiation decrease meristem size. Cell division is maintained in the proximal meristem by the presence of high auxin concentrations. Auxin activity is antagonized in the transition zone by cytokinin synthesized in the vasculature, which induces expression of SHY2 through the *AHK3/ARR1,ARR12* cytokinin signaling pathway. SHY2 represses auxin responses and represses PIN expression, limiting lateral auxin transport and enabling cell differentiation and elongation. SHY2 also regulates the cytokinin biosynthesis enzyme IPT5. SHY2 is itself regulated by auxin; it is marked for proteasome degradation through auxin-dependent recognition by the SCF^{TIR1} complex.

study [1], they explore further the role of auxin transport by PINs in meristem size regulation.

PIN1, PIN3 and PIN7 act in the root transition zone and are thought to influence root meristem maintenance [4,12]. Using quantitative real-time PCR and *PIN-GFP* fusion lines, Dello Ioio *et al*. [1] confirm expression of these three PIN proteins in the root vasculature and find that treatment with cytokinin markedly reduces their expression specifically in the vascular transition zone. Conversely, PIN expression is increased and expanded in *arr1* or *ahk3* mutant plants, in which cytokinin signaling is disrupted.

The region of the vasculature that loses PIN expression on cytokinin treatment coincides with the domain of SHY2 expression [1]. So does SHY2 modulate PIN expression in the transition zone? Dello Ioio *et al*. find that PIN expression is increased and expanded in the root vasculature of *shy2-31* (loss-of-function) plants whereas expression is decreased in *shy2-2* (gain-of-function) plants. SHY2 is also required for the cytokinin-mediated decrease in PIN expression. Together, these results indicate that SHY2 does indeed act to reduce PIN expression in the vascular transition zone.

SHY2 regulates meristem size throughout the transition zone

Increased PIN expression and meristem size are also observed in plants treated with exogenous auxin. Do these effects involve auxin-dependent degradation of SHY2? To test this, Dello Ioio *et al*. treated *shy2-2* plants with auxin [1]. The SHY2-2 protein does not interact with the auxin receptor

TIR1, and therefore accumulates, resulting in auxin resistance [13]. Auxin application did not trigger increases in PIN expression or meristem size in *shy2-2* plants, suggesting that SHY2 degradation does indeed contribute to these auxin effects. Similar experiments with gain-of-function mutants of other AUX/IAA family members will be needed to determine whether SHY2 is the only family member with this function.

The authors go on to investigate the possible involvement of SHY2 in auxin regulation of cytokinin biosynthesis [1]. Using an isopentyl transferase (*IPT5*) promoter-reporter fusion, they find that promoter activity of this cytokinin biosynthesis gene increases in the root vascular transition zone of plants treated with exogenous auxin and in the *shy2-31* loss-of-function mutant. In the gain-of-function mutant *shy2-2*, *IPT5* promoter activity and auxin responsiveness are abolished. These results demonstrate the existence of a second mechanism by which SHY2 links auxin and cytokinin pathways in the *Arabidopsis* root, although the details of this SHY2 negative feedback loop are not yet clear.

Putting these results together, Dello Ioio and colleagues [1] present a model for the mechanism of auxin antagonism by cytokinin through the auxin repressor SHY2 (Figure 2b), and they show the requirement for this circuit in positioning the transition zone between the meristem and the elongation differentiation zone. Their model accounts for the effects of exogenous auxin or cytokinin application on root meristem size, and explains the root meristem phenotypes observed in several auxin and cytokinin signaling mutants. They propose that auxin transport by the PIN efflux carriers directs auxin to a maximum near the stem-cell niche, where it effects auxin responses, maintains cell division and increases meristem size. Auxin responses are antagonized in the transition zone by the auxin-sensitive repressor SHY2, expression of which is induced by cytokinin synthesized in the transition zone vasculature. SHY2 accumulates here, where it represses expression of auxin-responsive genes, enabling differentiation of vascular cells.

SHY2 also effects differentiation of non-vascular cells in the transition zone. As found by Dello Ioio *et al.* [1], increases in PIN expression in the vasculature via auxin treatment or loss-of-function mutations in *arr1* and *shy2* are accompanied by increased meristem size in all cell files of the meristem. This intriguing result contributes to the model for SHY2-mediated regulation of meristem size. SHY2 repression of PIN expression in the vascular transition zone will ultimately limit lateral auxin transport. This deprives other cell types of the stem-cell fate signal - auxin - thus enabling differentiation of all cell types in the transition zone.

Auxin and cytokinin also act antagonistically in the specification of the root stem-cell niche. Here, auxin represses cytokinin action by activating expression of *ARR7* and *ARR15*, which encode A-type response-regulator proteins that repress cytokinin responses [5]. This raises the question of whether antagonistic auxin-cytokinin interactions are a common theme in the specification of cell identity. It is also interesting to consider the genes downstream of *SHY2* and *ARR1*, and how they might function to maintain stem-cell identity or enable differentiation. The results presented by Dello Ioio *et al.* [1] suggest that auxin-responsive genes in root tips are associated with stem-cell identity, whereas targets of ARR1 are predicted to be associated with differentiation processes. The results of this study could now inform the analysis of the large datasets of hormone-responsive genes collected from numerous genomic studies [14,15].

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